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Scientific editor O. Y. Hrynevych,
Doctor of Medical Sciences

PROTEFLAZID[®]: **clinical experience**

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The collection of scientific works describes the basics of the direct antiviral action of a wide range of the drug PROTEFLAZID® (drops) and dosage forms based on it – FLAVOVIR® (syrup) and PROTEFLAZID® (suppositories) in clinical conditions of use in viral infections caused by viruses of acute respiratory infections (including influenza and SARS-CoV-2); viruses: herpes, human papilloma, hepatitis (B and C) and mixed forms of viral and bacterial infections.

The scientific material and relevant conclusions are presented on the basis of many years of clinical experience in the use of the specified drugs with the involvement of systematic and meta-analyses, which demonstrates the evidence of the obtained clinical data in the treatment of diseases caused by various viral etiological factors, for the treatment of which practitioners used the drug PROTEFLAZID® or its medicinal forms (drops, syrup, suppositories).

A book for family doctors, pediatricians, therapists, infectious disease specialists and doctors of other specialties. The book will be interesting for everyone who cares about health and longevity both personally and for their relatives and friends.

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Section 1. Acute respiratory viral infections (ARVI)

1.1. Treatment and prevention of influenza with the use of boiflavonoids <i>O. Ye. Abaturov, I. L. Vysochina</i>	11
1.2. FLAVOVIR® in the prevention of infectious diseases in infants <i>O. V. Zubarenko, Yu. V. Desyatska, I. M. Shevchenko, N. Yu. Gornostaeva</i>	17
1.3. Acute respiratory infections in children, the latest trends in antiviral therapy <i>I. I. Andrikevich</i>	23
1.4. The efficacy of the preventive usage of flavonoids among the school age children in the complex reduction of the acute respiratory diseases incidence <i>I. L. Visochina, O. Ye. Abaturov</i>	30
1.5. Preventive and therapeutic effectiveness of boiflavonoids in children with recurrent respiratory infections <i>O. M. Mukvich, T. M. Kaminska</i>	37
1.6. FLAVOVIR® in pediatrics: a systemic analysis of the effectiveness and safety of using <i>G. V. Beketova, M. V. Khaytovych, O. Y. Hrynevych</i>	45
1.7. Analysis of the clinical trial results of the flavonoid effects on some cellular immunity indications in children and adults <i>G. V. Beketova, L. V. Kuznetsova, O. Y. Hrynevych, T. A. Broun, L. Ye. Momotiuk, O. M. Motuzka, L. O. Yashchenko</i>	55
1.8. PROTEFLAZID® vs COVID-19: successes in research <i>O. Y. Hrynevych, O. L. Panasiuk, S. P. Borshov, V. I. Matyash</i>	75
1.9. PROTEFLAZID® effectiveness for prevention and treatment of acute viral respiratory infections in the conditions of COVID-19 <i>O. Y. Hrynevych, S. P. Borshchov, V. I. Matyash, L. E. Momotiuk, O. M. Motuzka, L. O. Yashchenko</i>	83

Section 2. Herpes virus diseases (HVD)

2.1. PROTEFLAZID®: specific activity against herpes virus in preclinical investigations and its efficacy/safety in clinical practice (systemic review) <i>V. I. Matyash, O. Y. Hrynevych, O. L. Panasyuk, L. M. Solomakha</i>	105
2.2. PROTEFLAZID®: clinical experience in children of young and preschool age – systemic review of postmarketing surveillance <i>G. V. Beketova, O. Y. Hrynevych, L. M. Solomakha, N. I. Golovnia</i>	134
2.3. Meta-analysis of clinical trials results of efficacy and safety of the drug PROTEFLAZID® in the treatment and prevention of human herpes virus infection in children <i>S. O. Kramarev, V. S. Mykhailov, O. Y. Hrynevych, O. V. Vygovska, M. V. Lesnikova, L. A. Yashchenko, T. A. Broun</i>	149
2.4. PROTEFLAZID: specific activity in Epstein-Barr virus in a preclinical study, efficacy and safety in the clinic (systemic review) <i>O. Y. Hrynevych, S. O. Kramarev, V. I. Matyash, L. M. Solomakha, O. Vygovskaya</i>	162
2.5. Therapeutic efficacy and tolerability profiles of PROTEFLAZID®, suppository and drops among the patients with herpes virus infection in the exacerbation phase <i>A. G. Kornatskaya, O. O. Revenko, O. Y. Hrynevych, M. V. Shepetko</i>	174

Section 3. Papillomavirus infection (PVI)

3.1. PROTEFLAZID®: efficacy and safety in clinical practice for diseases caused by human papillomavirus (systematic review) <i>V. V. Kaminskiy, M. M. Shalko, L. I. Vorobyova, O. V. Romaschenko, O. Y. Hrynevych</i>	185
3.2. Evaluation of efficacy of PROTEFLAZID® in the treatment of HPV-infection: meta-analysis of long-term clinical trials results <i>V. V. Kaminskiy, M. N. Shalko, V. S. Mikhailov, M. V. Lesnikova, L. A. Yashchenko, O. Y. Hrynevych, T. A. Broun</i>	204
3.3. Comparative evaluation of drug use effectiveness in the therapy of cervical intraepithelial neoplasia (CIN) of mild to moderate degrees associated with genital papillomavirus infection <i>V. V. Kaminskiy, A. A. Sukhanova, M. N. Shalko, V. V. Sumenko, Y. V. Lavrenjuk</i>	215
3.4. The use of flavonoid glycosides in the treatment of genital HPV infection in women <i>V. M. Zaporozhyan, V. G. Marichereda, L. I. Dimcheva</i>	225
3.5. Comparative efficacy and safety of PROTEFLAZID® suppositories and drops in patients with cervical epithelial dysplasia caused by HPV infection <i>V. V. Kaminskiy, M. N. Shalko, O. Y. Hrynevych</i>	231
3.6. Clinical efficacy of topical use of vaginal suppositories PROTEFLAZID® in the treatment of cervical intraepithelial neoplasia of mild and moderate degree caused by the human papilloma virus <i>N. A. Godlevskaya, A. V. Starover</i>	237
3.7. The ways of reducing diagnostic and therapeutic aggression of the patients with HPV-infection in reproductive age <i>N. M. Voloshyna, E. D. Zvantseva</i>	247
3.8. Results of the clinical and molecular-histological assessment of the effectiveness of the drug PROTEFLAZID® in the treatment of papillomavirus infection in women with cervical intraepithelial neoplasia <i>V. O. Potapov</i>	259

Section 4. Mixed viral and bacterial infection (MVBI)

4.1. PROTEFLAZID®: treatment of herpesvirus and mixed infections. Meta-analysis of clinical trials results <i>V. I. Matyash, O. Y. Hrynevych, T. A. Broun</i>	271
4.2. Therapeutic efficacy of PROTEFLAZID® (suppository) among the patients with genitourinary viral-bacterial infection <i>V. O. Benyuk, O. Y. Hrynevych, I. A. Usevych, I. S. Sikachova, M. V. Shepetko</i>	292
4.3. Meta-analysis of clinical studies on the efficacy of flavonoids in viral and viral-bacterial diseases in children <i>S. O. Kramarev, O. Y. Hrynevych, O. B. Tonkovid, O. V. Vygovskaya</i>	300
4.4. PROTEFLAZID® and local immunity in diseases caused by human papillomavirus, herpesvirus and mixed urogenital infections <i>V. V. Kaminskiy, V. P. Chernyshov, O. Y. Hrynevych, V. O. Benyuk, A. H. Kornatska, M. N. Shalko, I. A. Usevych, O. O. Revenko, M. V. Shepetko, L. M. Solomakha</i>	310

Section 5. Viral hepatitis (VH)

5.1. Viral hepatitis: statistical analysis of clinical research on the effectiveness of using the drug PROTEFLAZID® <i>V. I. Matyash, O. Y. Hrynevych, L. O. Yashchenko</i>	321

- 5.2. PROTEFLAZID®: the specific activity against the hepatitis C virus in preclinical studies; efficacy and safety in the treatment of hepatitis B and C in clinical practice (systematic review)
A. M. Pechinka, O. Y. Hrynevych, T. A. Kryuchko, V. R. Shaginin, L. M. Solomakha 337

Section 6. Efficacy and safety in pregnant

- 6.1. PROTEFLAZID® in pregnant women: systematic review of observing of postregistration safety and efficacy
V. V. Kaminsky, M. N. Shalko, O. Y. Hrynevych 359
- 6.2. PROTEFLAZID®: a meta-analysis of clinical studies to assess the efficacy and safety of pregnant
V. A. Benyuk, Y. V. Kuvita, O. Y. Hrynevych, O. B. Tonkovid, I. A. Usevich, O. S. Neymark 369
- 6.3. PROTEFLAZID®: outcome analysis of clinical trials studying the impact of human herpesvirus infection on some cellular immunity indicators and gestation course in women
V. O. Benyuk, L. V. Kuznetsova, O. Y. Hrynevych, T. A. Broun, L. Ye. Momotiuk, O. M. Motuzka, L. O. Yashchenko 387
- 6.4. Prevention of Perinatal complications in pregnancy with chronic papilloma virus infection
S. M. Heryak, N. V. Petrenko, V. Yu. Dobryanska 400
- 6.5. Management of pregnancy in the association of HIV and herpes virus infection
V. V. Kaminsky, T. M. Anoshyna, T. V. Kolomiichenko 408
- 6.6. Application of PROTEFLAZID® for pregnant with associated toxoplasmic, herpetic, cytomegaloviral and Epstein-Barr viral infection
I. V. Lipkovska, V. H. Marychereda, I. L. Holovatiuk, O. P. Holovatiuk 414

Section 7. Pharmacoeconomics

- 7.1. PROTEFLAZID®: clinical and economic substantiation for use in therapy of herpes infection
I. A. Zupanets, T. S. Sakharova 423
- 7.2. The pharmacoeconomic basis of use of PROTEFLAZID® in the treatment of human papillomavirus
G. M. Voitenko, I. V. Kokhanov 432

Appendices

- PROTEFLAZID® (drops). Instruction for medical use of medicinal product 443
- PROTEFLAZID® (suppositories). Instruction for medical use of medicinal product 448
- FLAVOVIR® (syrup). Instruction for medical use of medicinal product 452
- National consensus on the management of patients with cervical intraepithelial neoplasia caused by human papillomavirus infection
V. M. Zaporozhan, V. V. Kaminsky, V. S. Svintsitskyi, V. O. Beniyk, N. M. Voloshyna, V. M. Honcharenko, I. T. Kyshakevych, I. V. Kovalchuk, A. H. Kornatska, V. H. Marichereda, V. F. Nagorna, V. O. Potapov, O. A. Revenko, V. V. Sumenko, L. V. Suslikova, K. V. Chaika, M. N. Shalko, M. O. Shcherbyna 456

Projects of SMC “Ecopharm” LTD

- honored by certificates of SEAL OF EXCELLENCE European Commission 475

In the early 2000s, the range of the antiviral agents was supplemented by the medicinal product PROTEFLAZID® (developed by SMC “Ecopharm” LTD, Kyiv, Ukraine) exerting direct antiviral, immunomodulating, anti-inflammatory, apoptosis-modulating and anti-oxidant effects; it had the important practical significance and was accompanied by the studies to evaluate its efficacy and safety in the various pathologies of viral etiology in the clinical settings.

Later, based on PROTEFLAZID® substance, three new substances and range of the dosage forms were developed: drops, syrup, suppositories and capsules. In 2018, the in-house production where the medicinal products based on PROTEFLAZID® substance were manufactured obtained GMP certificate. PROTEFLAZID® is a liquid ethanol extract made of the wild *Deschampsia caespitosa* L. and *Calamagrotis epigeios* L. grasses. The medicinal product has the original composition comprising the flavonoid aglycons (tricine, apigenin and luteolin) and their O- and C-glycosides contained in the matrix of the excipients – amino acids, carboxylic acids and sugars.

In 2000–2017, more than 230 clinical studies (controlled, comparative, randomized) were conducted; they confirmed the efficacy and safety of the range of medicinal products based on PROTEFLAZID® substance [PROTEFLAZID® (drops), PROTEFLAZID® (suppositories), FLAVOVIR® (syrup)]. The results of the clinical studies are presented as the articles in the specialized medical publications and are contained in this collection.

More than 22 thousand subjects took part in the specified clinical studies; more than 12 thousand of patients from the different age, social and other groups receive medicinal product PROTEFLAZID® for etiotropic treatment of viral diseases caused by RNA and DNA viruses.

Clinical studies formed the basis for a number of planned research and practical studies conducted by the research institutes of the Academy of Medical Sciences of Ukraine and health care institutions of the Ministry of Health of Ukraine. Some Ph. D. (38) and doctor's (9) theses were defended on this subject.

To confirm and summarize the scientific data obtained in the clinical settings and form the evidence base validating the efficacy and safety of medicinal product PROTEFLAZID®, in 2014–2021 four thematic meta-analyses, thirteen systemic reviews of the clinical observations and long-term results of the medicinal product use were performed and published in the scientific literature and presented in this collection.

Based on the accumulated clinical results of using medicinal product PROTEFLAZID® in the therapy of diseases caused by the viruses of influenza and ARVI, herpes, human papillomavirus, hepatitises and HIV-infection, the Ministry of

Health of Ukraine introduced some guidelines (20) and information letters “On innovations in the healthcare system” (29).

The clinical studies (controlled, comparative, randomized) determined and statistically confirmed that medicinal product PROTEFLAZID® is therapeutically effective at any stage of infection development but not only at the moment of virus replication unlike other antiviral products. In addition, the retreatment and long-term courses of using medicinal product PROTEFLAZID® do not cause the development of the resistant strains of the viruses and do not result in the immunosuppression/refractory conditions.

As for the influenza viruses, the detected property of medicinal product PROTEFLAZID® to not only inhibit the neuraminidase activity, but also, unlike other antiviral products of this class recommended for the treatment of influenza, to suppress the ability of the intracellular replication of influenza viruses by inhibiting the synthesis of virus-specific enzymes – thymidine kinase and RNA polymerase – is important, i.e. it has a poly-target mechanism of the direct antiviral action. Other significant difference of PROTEFLAZID® medicinal products from the influenza neuraminidase inhibitors (as in the structure of influenza incidence, it accounts for only 6 to 10% of patients with the ARVI) is that PROTEFLAZID® can inhibit the replication of RNA- and DNA-containing viruses due to the broad spectrum of the direct antiviral action.

A specific situation with the ARVI treatment has occurred in the recent years, when the pandemic coronavirus – SARS-CoV-2 and its varieties – has spread around the world. From February 2020 to WHO statement of pandemic, pharmacological company Ecopharm initiated its own humanitarian action to protect the medical staff in the clinics admitting SARS-CoV-2-infected patients and provided more than 30 thousand vials of medicinal product PROTEFLAZID® (drops) at no cost to more than 140 hospitals involved in providing care to the patients with COVID-19. Volume of charitable assistance and reports on the using the medicinal product PROTEFLAZID® obtained from the healthcare institutions allowed summarizing the experience of the preventive and therapeutic use of medicinal product PROTEFLAZID®. The National Academy of Statistics, Accounting and Audit of Ukraine analyzed the letters with reports from the healthcare institutions about the results of using medicinal product PROTEFLAZID®. The vast majority of the letters from the healthcare institutions outlined that the medical staff was in contact with potential COVID-19 patients and had the high risk of exposure to the coronavirus infection.

In total, 8 572 persons administered the medicinal product PROTEFLAZID® for preventive purpose in the specified period; they included 7 444 health care workers and 1 128 patients. The total number of persons who received the medicinal product PROTEFLAZID® for the purpose of treatment was 433 subjects. In total, 9 005 persons (including medical staff and patients) used the medicinal product PROTEFLAZID® (drops) for the purpose of prevention and treatment. The statistical parameter “Number of fatal cases” among the patients and staff of the healthcare institutions that provided such information was “0”.

In general, in comparison with the official data on COVID-19, among the persons who used the medicinal product PROTEFLAZID®: – there were no fatal cases; – proportion of sick medical staff in the healthcare institutions was decreased by 2.2;

– proportion of patients with the severe disease was decreased by 3.3; – mean treatment period was decreased by 1.8.

In 2019–2021 it was determined that the mechanism of the direct antiviral action of the medicinal product PROTEFLAZID® used in the acute respiratory viral infection associated with COVID-19 was attributable to: a) inhibiting activity of 3C-like protease (3CL protease) essentially important for replication of SARS-CoV-2 virus; b) inhibiting RNA polymerase needed for replication of SARS-CoV-2 virus; c) activation of Nrf2 transcription factor that reduces the expression of angiotensin-converting enzyme 2 receptors in the respiratory epithelial cells preventing the virus from entering the lung epithelial cells and suppressing SARS-CoV-2 virus replication protecting body cells from oxidative stress and inflammation, reducing the probability of the acute respiratory distress syndrome. That is, the medicinal product PROTEFLAZID® has a poly-target (multi-target) mechanism of the direct antiviral effect on SARS-CoV-2 coronavirus and protection of the body from the harmful effects of pathogenic processes caused by SARS-CoV-2 coronavirus.

Conducted and published results of the studies, meta-analyses and systemic reviews of the long-term clinical observations are the highest level of evidence of the results of clinical studies allowing us to state that the medicinal products based on the PROTEFLAZID® substance [PROTEFLAZID® (drops), PROTEFLAZID® (suppositories), FLAVOVIR® (syrup)] are the efficacious and safe original medicinal products demonstrating the direct antiviral effect in the infectious diseases caused by the RNA- and DNA-containing viruses.

Considering the extremely difficult situation with morbidity and mortality of the country's population caused by the COVID-19 pandemic, the company conducted the clinical study: "Multicenter, randomized, double-blind study of efficacy and tolerability of FLAVOVIR® (capsules) (SMC "Ecopharm" LTD, Ukraine) in the patients with the moderate COVID-19 receiving background therapy" in the pandemic conditions and during the war with the Russian fascism. FLAVOVIR® (capsules) is the innovation of the SMC "Ecopharm" LTD developed as a new dosage form based on the substance derived using the know-how technology from PROTEFLAZID® substance. Based on the obtained results, the superior efficacy of the medicinal product FLAVOVIR® (capsules) versus placebo was confirmed secondary to the standard background therapy in the patients with the moderate COVID-19. The results demonstrated the good therapeutic effect of medicinal product FLAVOVIR® (capsules) in the patients with COVID-19. Further studies conducted in this sphere will allow us to introduce a new effective medicinal product with the direct antiviral effect into the clinical practice of family medicine. This product will play an important role in the fight against such predicted viral threats as "Disease X".

As for the papillomavirus infection (PVI), it should be noted that Ukraine was the first country globally whose scientists managed to develop the medicinal product (PROTEFLAZID®) with the direct effect on the human papillomavirus (HPV) including its oncogenic types and prove its efficacy and safety in the treatment of patients with PVI and cervical dysplasia (cervical intraepithelial neoplasia, CIN) in the clinical studies. Preliminary data of the preclinical studies have shown that PROTEFLAZID® decreases the viral load and normalizes the mitotic activity of the cells transfected

with papillomavirus DNA by inhibiting the expression of papillomavirus proteins L1 and E7 concomitantly enhancing the expression of cellular proteins that are the products of the tumor suppressor genes. PROTEFLAZID® inhibits replication of the viral DNA, normalizes the mitotic activity of the cells transfected with papillomavirus DNA and enhances the expression of the tumor suppressor proteins (p53, Rb). Therapeutic effect of flavonoids (that are the components of PROTEFLAZID®) involves the combination of the antiproliferative, proapoptotic and direct antiviral effects indicating the poly-target mechanism of its action. Thus, the Ukrainian scientists and clinicians managed to significantly impact the course of the above mentioned precancer pathology and, as a consequence, facilitate the prevention of the cervical cancer that is one of the most prevailing and dangerous female malignant tumors and that is so important today, in the period of demographic crisis observed both in Ukraine and in the European countries.

**Sincerely,
President of SMC “Ecopharm” LTD**

Anatolii Matviiiovych Novyk



Section 1

1.1. TREATMENT AND PREVENTION OF INFLUENZA WITH THE USE OF BIOFLAVONOIDS

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Abstract

Based on the analytical review of the literature presented data on efficacy and safety of bioflavonoids (drug PROTEFLAZID® as a drops and syrup FLAVOVIR®) in the scheme of treatment and prevention of influenza. Analyzed in this paper scientific publications testify to the etiology and pathogenesis. Sound appointment PROTEFLAZID® mechanism influenza action is based on inhibition of the reproduction of the viruses of influenza including pandemic A/FM/1/47/H1N1/ by inhibition mechanism of neuraminidase activity of interferon synthesis induction.

Key words: influenza, children, flavonoids, treatment, prevention.

Introduction

The influenza is the most common disease in the history of humanity. The first official reference describing a flu-like condition is found in the writings of Hippocrates (412 BC). The next report about the flu-like epidemic is found in the works of the Roman historian Titus Livius as of 212 BC. Similar outbreaks of the disease in humans have been recorded in literature sources (1173 publications). The first documented influenza pandemic taking many lives was recorded in 1580.

The first European who tried to describe in detail the mechanism of influenza development was Dr. John Keys (Caius) (England, 16th century). The most devastating influenza pandemic in human history was the Spanish influenza (French: La Grippe Espagnole or Spanish: La Pesadilla). It killed 50 to 100 million people (2.6–5.2% of the world population) within 18 months in the season of 1918–1919 and infected about 400 million people (more than 20% of the world population) [9].

According to the official statistical reports, within the last century in the different countries worldwide (regardless of their economic development level), the total share of influenza, together with other acute respiratory viral infections, consistently accounts for up to 90% of the structure of human infectious diseases attracting the unflinching scientific, professional, economic, social and everyday interest to this problem.

There are a lot of terminological characterizations of influenza; their main essence is that influenza (flue) (from grieppe, grippen – “grip”, “seize”) is the acute infectious disease of the respiratory tract caused by the various types of influenza viruses and characterized by the intoxication and affection of the mucous membrane epithelium of the upper respiratory tract, mainly the trachea. According to the WHO protocols, influenza is the acute viral infection spreading easily between people; influenza circulates worldwide and can affect any person from any age

group; influenza causes the annual seasonal epidemics with its peak in winter in the temperate climates; influenza is a serious public health problem that causes severe illness and leads to the fatal cases in the high-risk populations and, finally, influenza epidemic may have negative effect on the economy by reducing labor productivity and involving the excessive burden on the public health services. According to the WHO estimations, during the global seasonal epidemics, 250 000 to 500 000 people die from influenza every year and during some epidemics the number of deaths can reach a million.

Influenza pathogen – RNA genome virus of Influenzavirus genus and Orthomyxoviridae family – is classified into three main types: type A influenza was studied and described in 1931–1936 by the group of scientists from the different countries including V. Smith, C. Andrewes, P. Laidlaw, A. A. Smorodyntsev, L. A. Zilber, etc.; in 1940, scientists T. Francis and T. Medgill verified type B influenza and, finally, type C influenza was identified by R. Taylor in 1947 [7, 8, 9].

The outer envelope of the influenza virus consists of two main glycoproteins. One of which has hemagglutinating (H) activity and the other one – neuraminidase activity (N). Influenza virus surface antigens – hemagglutinin and neuraminidase – play an important role in the immune response formation in influenza. Hemagglutinin ensures the ability of the virus to bind to the cell and neuraminidase is responsible, firstly, for the ability of the viral particle to enter the host cell and, secondly, for the ability of the viral particles to exit the cell after replication. The intrinsic proteins surrounding RNA compose the S-antigen and define the virus type (A, B or C), the surface antigens – hemagglutinin and neuraminidase – compose V-antigen and define the virus subtype (e.g., H1N1, etc.) [7, 8].

Characteristic feature of influenza viruses is their ability for antigenic variation. It may involve the “drift” (partial variation of antigenic determinants) or the “shift” (complete replacement of a genome fragment encoding the synthesis of hemagglutinin or neuraminidase). Drift is most common in type A influenza but it is also met in type B influenza. The antigenic shift is a specific feature of type A influenza leading to the emergence of its new types. High variability of influenza viruses explains the unpredictability of disease epidemics. Type C virus is less variable and causes only small epidemic outbreaks. Today, more than 2 000 antigenic variants of the influenza virus have been studied and known.

After E. Pasquier described the influenza in 1403 of the last century, 18 influenza pandemics were officially recorded [7]. The shift variants of the type A influenza virus that formed the epidemics in 19th – 21st centuries are provided in the Table.

Having caused the pandemic in 2009, today A(H1N1) virus [7, 8, 9] continues to circulate in the human population as one of the seasonal influenza viruses. According to the official data of the Ministry of Health of Ukraine, since the beginning of the 2015–2016 epidemic season, more than 3.2 million patients with influenza and ARVI have been recorded in Ukraine accounting for 8.3% of the population and 62.8% of the total number of patients were children under 17 years of age. In general, influenza and ARVI incidence is now 41.4% higher than in the same period of the previous epidemic season. Since the beginning of the current epidemic season, 220 laboratory-confirmed fatal cases of influenza have been recorded to date (34 cases were identified as type A influenza virus, atypical; 2 cases – type B influenza virus; 183 cases – type H1N1 influenza virus [swine influenza]), there were three children under 17 years and one pregnant among the deceased persons. According to the official data, in Ukraine, at the height of the seasonal increase of the influenza and ARVI incidence, tens of thousands of people fall ill every day, about half of them are children under 17 years old [9].

Table. The shift variants of the type A influenza virus that formed the epidemics in 19th – 21st centuries

Year	Subtype	Spread
1889–1890	H2N8	Severe epidemic
1900–1903	H3N8	Moderate epidemic
1918–1919	H1N1	Severe epidemic (Spanish influenza), lethality: 50 to 100 million people
1933–1935	H1N1	Medium epidemic
1946–1947	H1N1	Medium epidemic
1957–1958	H2N2	Severe epidemic (Asian influenza), about 2 million deaths
1968–1969	H3N2	Moderate epidemic (Hong Kong influenza), about 1 million deaths
1977–1978	H1N1	Moderate epidemic, children and adults under 25 years old were sick, 300 thousand persons died
1995–2010	H5N1	Sporadic cases (avian influenza), a few thousand persons fell ill with influenza with 50% lethality due to complications
2009–2010	H1N1	Moderate epidemic (swine influenza), lethality in 2009 was 100 to 400 thousand people

According to the WHO Regional Office for Europe [9], H1N1 accounts for about 80% of all type A influenza viruses in the current season; moreover, the influenza was widespread in Finland, Sweden, Switzerland, Norway, Estonia, Netherlands, Ireland, Portugal, Greece, Slovenia, Georgia, Kazakhstan, Israel. According to ECDC data, in the current season, in the structure of influenza and AVRI incidence, A(H1N1)pdm09 influenza is prevailing both in Europe and USA where according to the CDC data, at the beginning of 2016 calendar year, the rate of seeking care due to influenza and ARVI was 2.2%, and the mortality rate due to influenza was 6.8%.

At the current stage of society development, influenza epidemics [7, 8, 9] are observed almost every year and their characteristic feature is that two or three representatives of influenza viruses can participate in the epidemic process simultaneously or sequentially (H1N1, H3N2 and B). Influenza pandemics, regardless of their severity, affect a significant part of the population and present a significant burden on the healthcare system of any country in the world. The effective instrument of control and unification of the treatment and diagnostic process in the world medicine at the modern stage is adherence to the influenza treatment protocols developed on the basis of evidence-based medicine. In Ukraine, the valid regulatory document is protocol No. 499 of the Ministry of Health of Ukraine; a special place in its guidelines for influenza treatment is given to the antiviral agents – neuraminidase inhibitors that selectively inhibit the activity of influenza A and B virus neuraminidases responsible for the release of the new viral particles from the infected respiratory tract cells [7, 8, 9].

The availability of a number of side effects of the new generation antiviral agents – Zanamivir and Oseltamivir – initiates the search for a safer and more efficacious product to control the seasonal influenza epidemics with the sufficient evidence base of studies confirming its ability to inhibit the reproduction of type A and B influenza viruses due to the mechanism of inhibition of their neuraminidase activity, as the main requirement of the protocol medicine today, which led to the analytical review of the medical literature on the above mentioned problem.

Based on the results of the analysis of scientific articles published within the last years, the solution of the task was to confirm the existence of the evidence base pertinent to the efficacy and safety

of the bioflavonoids (medicinal product PROTEFLAZID[®], drops and *FLAVOVIR[®], syrup) used in the influenza treatment and prevention regimen.

Analyzed results of the research studies presented in the periodical publications indicate that prescription of medicinal product PROTEFLAZID[®] in influenza may be considered as etiologically and pathogenetically justified; its mechanism of action is associated with its ability to inhibit the neuraminidase activity of the influenza viruses, which is consistent with the current guidelines of the unified influenza treatment protocol, in which the main indication for prescribing the etiotropic and pathogenetic therapy is the selective inhibition of the activity of neuraminidase of type A and B influenza viruses available in the recommended medicinal product.

The report of the preclinical study of the new (dosage) forms of medicinal product PROTEFLAZID[®] conducted on models of influenza viruses at L. V. Hromashevskiy Institute of Epidemiology and Infectious Diseases of the Academy of Medical Sciences of Ukraine (2006) contains the convincing evidence that bioflavonoids of medicinal product PROTEFLAZID[®] exert the anti-influenza effect based on the inhibition of influenza virus reproduction due to the mechanism of inhibition of neuraminidase activity, induction of interferon synthesis and inhibition of synthesis of RNA of phage T7 DNA-dependent RNA polymerase.

In 2010, under the guidance of the Academy of Medical Sciences of Ukraine, L. V. Hromashevskiy Institute of Epidemiology and Infectious Diseases, the study was conducted to investigate the pharmacodynamic properties and specific antiviral activity of medicinal product PROTEFLAZID[®] on *in vitro* and *in vivo* models [1, 3]. It has been proven that flavonoids contained in FLAVOVIR[®] syrup inhibit the replication of viral DNA and RNA, including influenza viruses both *in vitro* and *in vivo*. The mechanism of the antiviral effect of the medicinal product involves the virus-specific enzyme inhibition – DNA polymerase, thymidine kinase and reverse transcriptase; anti-influenza effect – inhibition of neuraminidase activity, induction of the endogenous α - and γ -interferon synthesis, inhibition of RNA virus synthesis. Potentiating the action of the apoptosis-inducing substances, the medicinal product promotes faster elimination of virus-infected cells and prevention of the chronic diseases caused by the latent viral infections [1, 3].

In 2010, the study was conducted to investigate the efficacy of flavonoids towards the pandemic agent of type A influenza on the animal model of influenza pneumonia. Study results demonstrated that the medicinal product reduced the infectious titer of influenza virus (H1N1/California) in the lung tissues of infected animals by 250 times [6]. The results of the clinical studies proved that when the medicinal product FLAVOVIR[®] is used for a long time, it does not inhibit the activity of α - and γ -interferon production resulting in the normalization of the patient's immune status [6].

A group of authors from the Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine together with the researchers from L. V. Hromashevskiy Institute of Epidemiology and Infectious Diseases of the Academy of Medical Sciences of Ukraine (2013) conducted an experiment and detected and confirmed the availability of the inhibiting effect of the herbal extract of *D. caespitosa* L. and *C. epigeios* L. on the synthesis of RNA and DNA viruses *in vitro*. All test-agents were found to be effective inhibitors of RNA and DNA virus replication. The above mentioned herbal components is the basis of the medicinal product PROTEFLAZID[®] allowing to state that this medicinal product has the direct antiviral properties [3, 10].

In 2014, a group of authors from P. L. Shupyk National Medical Academy of Postgraduate Education, O. O. Bogomolets National Medical University, State Agency on Science, Innovation

*Editor note: in 2018, the trade mark IMUNOFLAZID[®] was replaced with the trade mark FLAVOVIR[®], therefore, in this and following articles, the syrup name IMUNOFLAZID[®] was replaced with FLAVOVIR[®] syrup

and Informatization (Kyiv, Ukraine) performed the systemic analysis of the efficacy and safety of FLAVOVIR® used in pediatrics including the analysis of the results of 12 clinical studies involving more than 800 children. Based on the results of the analytical review it was confirmed that the medicinal product FLAVOVIR® (syrup) used at the usual therapeutic doses has the direct antiviral action and causes the immunomodulatory effect, reduces the level of the pro-inflammatory cytokines, stimulates T-helper activity, increases the level of protection reducing the development of oxidative stress [10]. It was demonstrated that the retreatment courses with the medicinal product FLAVOVIR® (syrup) used in the recurrent viral infections had the high efficacy.

Clinical efficacy of the preventive use of FLAVOVIR® syrup was confirmed by 1/3 decrease of influenza and ARVI incidence in the school age children, 35% minimization of sick days number in the child care institutions, 2.2–2.3 decrease of ARVI incidence in the younger children and prevention of the severe disease. Many researchers also noted the high safety profile of FLAVOVIR® syrup daily used in children for a long time [2]. Analysis of the results of 30 clinical studies of flavonoids efficacy in the viral and viral and bacterial diseases in 2 699 children performed by the group of scientists from O. O. Bogomolets National Medical University, State Agency on Science, Innovation and Informatization of Ukraine and P. L. Shupyk National Medical Academy of Postgraduate Education (Kyiv, Ukraine) in 2014 also demonstrated the efficacy and safety of flavonoids (PROTEFLAZID®, FLAVOVIR®) used in children [4].

Conclusions

Summarizing the results of the above mentioned systematic review, we made a conclusion that the therapeutic effects of FLAVOVIR® syrup give ground to recommend it for the widespread use in the children suffering from influenza and acute respiratory viral infections. FLAVOVIR® syrup is the medicinal product completely meeting the requirements to the prevention and treatment of ARVI in children [2, 5, 11, 12].

Therefore, the medicinal products PROTEFLAZID® (drops) and FLAVOVIR® (syrup) containing the bioflavonoids may be recommended to control the present-day seasonal influenza epidemics as etiotropic and pathogenetic medicinal products used both in treatment regimens and for prevention in patients regardless of their age, taking into consideration the evidence base of their clinical efficacy and high safety profile and definition of the mechanism of anti-influenza action, which is based on the suppression of influenza virus reproduction due to the mechanism of inhibition of neuraminidase activity and promotion of interferon synthesis..

References:

1. Pal'chikov's'ka G., Vasil'chenko O., Platonov M., Starosila D., Porva Yu., Rimar S., Atamanyuk V., Samylenko S., Ribalko S. (2013) Antivirusni vlastivosti roslinnih flavonoïdiv – ingibitoriv sintezu DNK i RNK [Antiviral properties of herbal flavonoids – inhibitors of the synthesis of DNA and RNA]. Biopolymers and cell, vol. 29, no 2, pp. 150–156.
2. Beketova G., Hajtovich N., Hrynevych A. (2014) Immunoflazid v pediatrii: sistemnyj analiz effektivnosti i bezopasnosti primeneniya [Immunoflazid in pediatrics: a systematic review of the effectiveness and safety of use]. Pediatriya. Vostochnaya Evropa, vol. 3, no 07, pp. 141–152.
3. Zvit pro doklinichne vivchennya novih (Hkuval'nih) form Proteflazidu na modelyah vu'usu gripu (2006) [Report on preclinical study of new (medical) forms of Proteflazid on models of influenza virus].
4. Kramar'ov S., Grinevich O., Tonkovid O., Vigov's'ka O. (2014) Meta-anaHz rezul'tativ klinichnih doslidzhen' effektivnosti flavonoïdiv pri virusnih ta virusno-bakterial'nih zahvoryuvannyah u ditej

- [Meta-analysis of clinical studies on the efficacy of flavonoids in viral and Wrubel diseases in children]. *Sovremennaya pediatriya*, vol. 5, no 61, pp. 1–7.
5. Nazarenko V., Ovchinnikova N. (2009) Dosvid vikoristannya preparatu Imunofl azid u Hkuvanm ta profilaktici gripu i gostrih respiratornih infekcij [Experience in the use of the drug Immunoflazid in the treatment and prevention of influenza and acute respiratory infections]. *Sovremennaya pediatriya*, vol. 23, no 1, pp.1–2.
 6. Otchet o NIR E'ksperimental'noe izuchenie protivovirusnoj e'fektivnosti preparata Proteflazid v otnoshenii vzbuditelya grippa A (H1N1) na modeli grippoznoj pnevmonii u zhivotnyh (2010) [The research report Experimental study of the antiviral efficacy of the drug Proteflazid against the pathogen of influenza A (H1N1) on the model of influenza pneumonia in animals] Ministerstvo zdorov'ya Rossijskoj Federacii, Nauchno-issledovatel'skij Institut grippa.
 7. Kaverin N. (2010) Pandemii grippa v istorii chelovechestva [Flu pandemic in the history of mankind]. Available at: <http://www.polit.ru/article/2011/02/07/pandemia/>.
 8. Rybalko S. (2010) Sovremennoe sostoyanie problemy grippa A [Current state of the problem of influenza A]. *Zdorov'ya nacu*, vol. 15, no 3, pp. 169–178.
 9. (2016) Sezonnyj gripp A(H1N1): otvety na glavnye voprosy. Evropejskoe regional'noe bjuro VOZ [Seasonal influenza A(H1N1): the answers to the main questions. The European regional office of WHO, January 2016]. Available at: <http://www.euro.who.int/ru/about-us>.
 10. Sokurenko L. (2012) Antitoksichna diya Imunoflazidu pri gripr Problemi ekologichnoi ta medichnoi genetiki i klimchnonoi imunologii, vol. 6, pp. 408–413.
 11. Tokarchuk N., Starinec' L. (2012) Vikoristannya Imunoflazidu dlya profilaktiki ta likuvannya gripu i GRVI u ditej pid chas sezonного pidvishhennya zahvoryuvanosti [Use Immunoflazid for the prevention and treatment of influenza and ARVI in children during the seasonal rise of morbidity]. *Sovremennaya pediatriya*, vol. 41, no 1, pp. 123–127.
 12. Tokarchuk N., Starinec' L. (2012) Dosvid vikoristannya Imunoflazidu v kompleksi Hkuval'no-profilaktichnih zahodrv pid chas sezonного pidvishhennya zahvoryuvanosti na grip ta GRVI [Experience in the use of Anoplasy in the complex of treatment and prophylaxis during seasonal increase in the incidence of influenza and SARS]. *Perinatologiya i pediatriya*, vol. 49, no 1, pp. 1–5.

1.2. FLAVOVIR® IN THE PREVENTION OF INFECTIOUS DISEASE IN INFANTS

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Abstract

The aim of this work was to evaluate the efficacy and safety FLAVOVIR® for the prevention of infectious diseases in children in the first year of life. Methods. A comparative controlled study in parallel groups of healthy newborns. According to the results of randomization, patients were divided into 2 groups. The study group included 39 children who are over 14 days of receiving the FLAVOVIR® per day. The control group consisted of 36 infants who did not receive the drug. The effectiveness of preventive measures was assessed by the absence of disease over a period of six months follow-up. The Results. Studies have shown that children of the main group in the 4-fold decreased the incidence of infectious diseases, the relative risk of disease decreased by 75%, the chances of the disease decreased to 0.03. During the administration of the drug is not registered a single case of side effects. Conclusions. FLAVOVIR® – an effective and safe means of preventing infectious diseases in children in the first year of life.

Key words: FLAVOVIR®, the children of the first year of life, prevention of infectious diseases

Introduction

The epidemiological studies conducted in some European countries indicate that on average, every child has 3 to 6 episodes of the acute viral respiratory infection (ARVI) per year [7, 8, 13]. The high incidence of ARVI is explained by the easy invasion and high antigenic variability of viruses, their ability to persist in the child's body secondary to the immature immune system and the presence of many serotypes of the infectious pathogens. Viral infections are prevailing among the respiratory diseases in children. Total number of viruses causing ARVI is more than 200. Mixed viral and viral and bacterial respiratory infections are also of great importance [1, 4, 9].

In addition to the direct etiological causes of ARVI, some factors facilitating the development of recurrent episodes of the respiratory infection in children should also be mentioned; they include such important aspects as unfavorable premorbid background, perinatal central nervous system damages, intrauterine infection, immaturity, prematurity, etc. and have negative effect on the systems of the developing body and especially on the full development of immunity [1, 7, 8].

The physiological (critical) periods of the development of a child's immune system are of considerable importance for children's susceptibility to ARVI, as they largely determine both the course and consequences of childhood diseases. The first critical period is up to 28 days

of life, the second one – up to 4–6 months, the third one – up to 2 years, the fourth one – up to 4–6 years, the fifth one – up to 12–15 years.

The child is the least protected in the first two critical periods. It is explained by the fact that in the first critical period (neonatal period), a child's immunity is passive, as it is ensured exclusively by the maternal antibodies when the endogenous immunity is in the suppression stage. Characteristic features of this period are disposition to generalization of the process, septic conditions and high sensitivity to the viral infections. During the second critical period (4–6 months), the maternal antibodies are destroyed and the developing primary immune response is ensured by IgM synthesis and does not leave immunological memory [11]. The insufficiency of the local immune system is manifested by the recurrent ARVI, intestinal infections and skin diseases. Thus, the age from 0 to 6 months is characterized by the increased sensitivity of a child to ARVI and is the characteristic feature of its ontogenesis. If the ARVI is prolonged and complicated, the immune system and anti-infective defense mechanisms are incapable – when “the viruses impose a sentence and bacteria execute it” [2, 11].

There is a continuing trend towards the increased complications caused by infections, especially in the first six months of a child's life necessitating the search for the effective and safe methods of their treatment and prevention aimed at preventing and correcting the deviations of the children's health [1, 7, 9]. When choosing the ARVI therapeutic approach in the young children, special attention is paid to the safety of medicinal products, their pharmacodynamic properties to suppress the activity of viral infection and increase the nonspecific immunoprotection of the body both at the local and systemic levels [4, 8, 13].

The studies of the antiviral, antioxidant and immunomodulatory properties of flavonoids have been conducted globally for a long time. The flavonoids comprise a group of the natural biologically active compounds – benzopyrone derivatives. The results of the experimental studies and clinical trials confirmed the efficacy of the flavonoids in the prevention of the respiratory, immune, cancer, neurodegenerative, cardiovascular and other diseases. Considering it, one of the promising areas in the treatment of ARVI in children is the use of the medicinal products from the group of bioflavonoids. In particularly, efficacy and safety of the domestic antiviral product from the group of bioflavonoids – FLAVOVIR® syrup (SMC “Ecopharm” LTD) has been studied within the last 10 years. Each 100 ml of syrup contain 2 ml of PROTEFLAZID® liquid extract derived from the mixture (1:1) of *Herba Deschampsia caespitosa* L. and *Herba Calamagrostis epigeios* L. grasses; 1 ml of extract contains not less than 0.32 mg/ml of flavonoids equivalent to rutin. The flavonoids contained in FLAVOVIR® syrup belong to the groups of flavone and flavonol glycosides. It was demonstrated that flavonoids contained in the medicinal product FLAVOVIR® inhibit replication of the DNA and RNA of respiratory viruses, including influenza, both *in vitro* and *in vivo*. The mechanism of the antiviral action of the medicinal product involves the virus-specific enzyme inhibition – DNA and RNA polymerases, thymidine kinase and reverse transcriptase; anti-influenza action – inhibition of neuraminidase activity, inhibition of viral RNA synthesis and induction of endogenous α - and γ -interferon synthesis. By enhancing the apoptosis processes, the medicinal product promotes faster elimination of virus-infected cells and prevention of the chronic diseases associated with the latent viral infections. The results of the clinical studies demonstrated that when FLAVOVIR® syrup is used daily for a long time, it does not inhibit the activity of α - and γ -interferon production resulting in the normalization of the patient's immune status [5, 10, 12, 15]. FLAVOVIR® protects the mucous membranes of

the upper respiratory tract normalizing the local immunity parameters (lactoferrin, sIgA and lysozyme); enhances the antioxidant status of cells by inhibiting the free-radical processes and preventing the accumulation of the lipid peroxidation products, decreases the intoxication, promotes the body recovery after the infection and adaptation to the adverse environmental factors [10, 14].

The clinical studies of FLAVOVIR® syrup conducted in the different infectious diseases demonstrated its high clinical and immunological efficacy and absence of the side effects. Having the high safety profile, FLAVOVIR® is allowed to be used in children from birth [8, 10, 14].

Study objective: evaluate the efficacy and safety of the medicinal product FLAVOVIR® (syrup) used for prevention of the infectious diseases in children under 12 months.

Study materials and methods

A comparative controlled study in parallel groups of healthy newborns whose mothers were carriers of TORCH infections was conducted in the neonatal department of Odesa Maternity Hospital No. 7. Relatives of all patients received oral information about all study procedures and provided informed consent to take part in the study. Based on the randomization, the patients were divided into two groups. The study group (39 children) received FLAVOVIR® syrup at 0.25 ml (half of therapeutic dose) twice daily as directed within 14 days after the birth. The control group consisted of 36 infants who did not receive the medicinal product.

After the discharge from the Maternity Hospital, all children had the 6-month outpatient follow-up; their diseases were recorded in the medical records. The effectiveness of the preventive measures was assessed by the absence of morbidity during the follow-up period.

There were no statistically significant clinical differences between the groups. Mean maternal age in the study group was 26.57 ± 1.32 years, in control group – 25.73 ± 1.41 years. All children in the study group were full-term; the mean gestational age was 38.44 ± 0.59 weeks. The mean weight of newborns was 3101.81 ± 166 g, body length – 49.18 ± 1.09 cm, head circumference – 33.15 ± 0.87 cm, chest circumference – 32.81 ± 0.73 cm. Mean Apgar score at the first minute of life was 7.81 ± 0.15 , at the fifth minute – 8.31 ± 0.23 .

All children from the control group were also full-term; the mean gestational age was 39.05 ± 0.39 weeks. The mean weight of newborns was 3263.82 ± 154.50 g, body length – 49.87 ± 0.69 cm, head circumference – 33.38 ± 0.76 cm, chest circumference – 33.11 ± 0.59 cm. Mean Apgar score at the first minute of life was 7.64 ± 0.18 , at the fifth minute – 8.50 ± 0.18 .

Statistical analysis of the obtained data was performed using Statistica 8.0, MedCalc 14.8.1 and Microsoft EXCEL 2010 with integrated add-on AtteStat 12.5 and internet-calculator SISA (Simple Interactive Statistical Analysis). The mean sample values of quantitative variables are given in the text as $M \pm m$, where M is the mean sample value and m is the error of mean. Proportions (percentages) are presented as 95% confidence intervals. In all statistical analysis procedures when testing null hypotheses, the critical significance level p was equal to 0.05. The study of the relationship between pairs of discrete qualitative variables was performed using the analysis of paired contingency tables where the following was evaluated: the value of Pearson test, chi-square (χ^2), significance level achieved (p), odds ratio (OR), values of the relative risk reduction (RRR) and number needed to treat (NNT) (number of patients that need to receive a treatment for one person to experience a positive effect that wouldn't have occurred otherwise) with determination of 95% confidence intervals. Kramer coefficient, ϕ was used to assess the strength of association between two qualitative variables [3, 6].

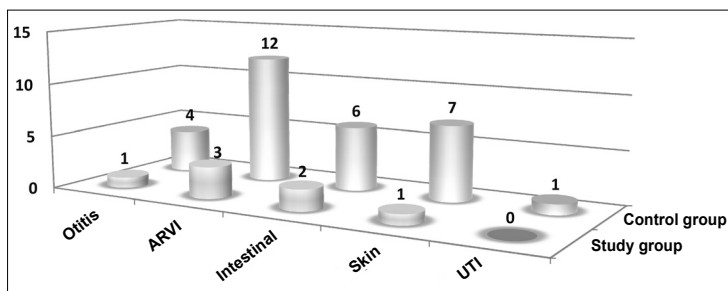


Figure 1. Distribution of infections depending on the localization

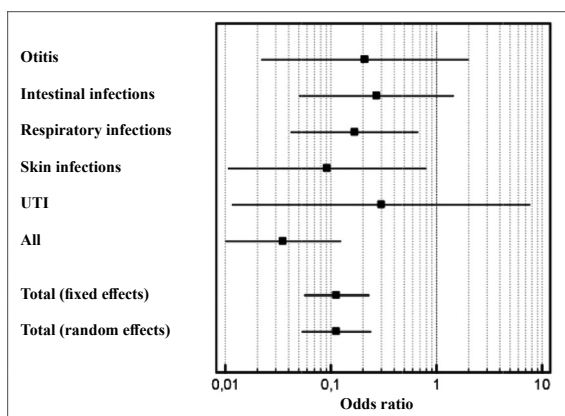


Figure 2. Effectiveness of preventing infectious diseases in children who received FLAVOVIR® syrup

Study results and their discussion

The subjects from the study group included 21 (53.84%, 95% confidence intervals (CI) 38.35–69.64) boys and 18 (46.14%, 95% CI 30.35–61.64) girls. Control group included 17 (47.22%, 95% CI 30.69–63.3) boys and 19 (52.78%, 95% CI 36.69–69.3) girls.

Retrospective analysis of the children's health within 6 months of life showed that only 7 children (17.94%, 95% CI 5.94–30.05) fell ill in the study group. Among them there was one case of each diagnosed disorder: acute otitis media (2.56%, 95% CI –2.57–8.57) and streptoderma (2.56%, 95% CI –2.57–8.57), two children had the intestinal infection (5.12%, 95% CI –1.75–13.75) and three children had ARVI (7.69%, 95% CI –0.86–16.86).

The disease incidence in the control group for the same period was 4.3 times higher – 30 children (83.33%, 95% CI 70.72–95.27) fell ill. Based on the nosiforms, the patients were distributed as follows: acute catarrhal otitis media – 4 children (11.11%, 95% CI 0.77–21.22), intestinal infections caused by opportunistic microflora (*Proteus mirabilis*, *Campilobacter coli*, *Enterobacter aerogenos*) – 6 children (16.67%, 95% CI 4.72–29.27), ARVI – 12

(33.33%, 95% CI 17.64–48.36), skin infections – 7 (19.44%, 95% CI 5.4–32.6), urinary tract infections – 1 child (2.77%, 95% CI –2.59–8.13). See the graphical data in Figure 1.

The comparison of the incidence in the studied groups showed the statistically significantly decreased incidence in the study group of children who received FLAVOVIR®: $\chi^2 = 32.12$, RRR – 79% (95% CI 61–88%), OR – 0.03 (95% CI 0.01–0.14), NNT – 2 (95% CI 1–2), Kramer coefficient $\phi = -0.68$, $p = 0.0001$. FLAVOVIR® showed to be the highly effective product to prevent the respiratory infections: $\chi^2 = 7.63$, RRR – 77 (95% CI 21–94), OR – 0.16 (95% CI 0.03–0.73), NNT – 4 (95% CI 3–20), Kramer coefficient $\phi = -0.32$, $p = 0.006$. Also, the statistically significant differences in the groups were observed in the skin infections: $\chi^2 = 6.85$, RRR – 88 (95% CI 17–99), OR – 0.09 (95% CI 0.01–0.8), NNT – 5 (95% CI 4–44), Kramer coefficient $\phi = -0.3$, $p = 0.009$.

The evaluation of quality of prevention of the other infection types showed the lower efficacy of the product. In the intestinal infections ($\chi^2 = 2.6$, CMP – 69 (95% CI –67–95), OR – 0.27 (95% CI 0.03–1.65), NNT – 9 (95% CI 5–), Kramer coefficient $\phi = -0.18$, $p = 0.1$), otitis media and urinary tract infections, no statistically significant differences were observed in the studied groups of children. The results of comparison performed in groups are presented in Table 1 and Figure 2.

Table 1. Evaluation of efficacy of prevention of infectious diseases in young children who received FLAVOVIR®

Infection localization	RRR (95% CI)	OR (95% CI)	NNT (95% CI)	P
Otitis media	77 (–108–99)	0.21 (0.01–2.18)	11 (7– ∞)	0.14
Upper respiratory tract diseases	77 (21–94)	0.16 (0.03–0.73)	4(3–20)	0.006
Intestinal infections	69 (–57–95)	0.27 (0.03–1.65)	9(5– ∞)	0.1
Skin infections	88 (17–99)	0.09 (0.01–0.8)	5(4–44)	0.009
Urinary tract infection	100 (–14.93–100)	0.00 (0.00–16.3)	36(36– ∞)	0.29
All recorded infections	79 (61–88)	0.03 (0.01–0.14)	2 (1–2)	0.0001

All diseases observed in the study group of followed-up children (Fig. 1) were exclusively mild; mean duration of disease was 6 ± 0.7 days. No side effects associated with FLAVOVIR® were observed in children from the study group. Moderate infections were recorded in 30% of children from the control group. There were no severe infections. Mean disease duration was 8.25 ± 0.45 days.

Conclusions

1. The results of the conducted study demonstrated the high efficacy of medicinal product FLAVOVIR® (syrup) in prevention of the infectious diseases in infants.

2. FLAVOVIR® syrup used for prevention of the infectious diseases in children from the birth by following regimen: 0.25 ml (half of therapeutic dose) twice daily (as directed) for 14 days allows reducing incidence, duration and severity of the infectious diseases in infants.

3. Medicinal product FLAVOVIR® (syrup) used in clinical settings is well tolerated by children and does not cause the side effects confirming its high safety profile.

References:

1. Зайцева О. В. Лікування та профілактика гострих респіраторних інфекцій у дітей, що часто хворіють / О. В. Зайцева, С. В. Зайцева // Лікуючий лікар. – 2008. – №8. – С. 53–57.
2. Іванова В. В. Стан клітинного та гуморального імунітету при інфекціях у дітей та його регуляція за допомогою імуномодуляторів / В. В. Іванова, Г. Ф. Железнікова, І. В. Шилова // Зб. наук. статей. – М., 2006. – С. 4–18.
3. Новіков Д. А. Статистичні методи в медико-біологічному експерименті (типові випадки) / Д. А. Новіков, В. В. Новочадов. – Волгоград: Вид-во ВолДМУ, 2005. – С. 84
4. Овсяннікова Є. М. Роль протизапальної терапії в лікуванні гострих респіраторних захворювань у дітей / Є. М. Овсяннікова, Н. А. Коровіна // УСошІшт Medicum. Педіатрія. – 2007. – №2. – С. 10–12.
5. Панасюк О. Л. Етіопатогенетична терапія герпесвірусної інфекції із застосуванням протефлазиду та ультрафіолетового опромінювання крові: автореф. дис. ... канд. мед. наук. : 14.01.13 / О. Л. Панасюк. – К. – 180 с.
6. Реброва О. Ю. Статистичний аналіз медичних даних. Застосування пакету прикладних програм STATISTICA/О. Ю. Реброва. М. : Медіасфера, 2002. – С. 312.
7. Резніченко Ю. Г. Пошук шляхів профілактики гострих респіраторних захворювань у дітей – мешканців великого промислового міста / Ю. Г. Резніченко, Р. Л. Шевченко, В. І. Бессікало // Сучасна педіатрія. – 2008. – Т. 19, №2. – С. 49–50.
8. Романцов М. Г. Діти, що часто хворіють: сучасна фармакотерапія: кер. для лікарів / М. Г. Романцов. – М., 2006. – 189 с.
9. Самсигіна Г. А. Діти, що часто хворіють: проблеми патогенезу, діагностики та терапії / Г. А. Самсигіна // Педіатрія. – 2005. – №1. – С. 66–73.
10. Токарчук Н. І. Досвід використання Імунофлазиду в комплексі лікувально-профілактичних заходів під час сезонного підвищення захворюваності на грип та ГРВІ / Токарчук Н. І., Л. С. Старинець // Перинатол. та педіатрія. – 2012. – Т. 49, №1. – С. 1–5.
11. Фрейдлін І. С. Особливості імунітету у дітей різного віку [Електронний ресурс] / І. С. Фрейдлін. – Режим доступу: <http://www.licopid.ru/osobennosti-immuniteta-u-detey-raznogo-vozrasta>. – Назва з екрану.
12. Експериментальне вивчення протівірусної ефективності препарату Протефлазид щодо збудника грипу А (H1N1)v на моделі грипозної пневмонії у тварин: звіт про НДР / Міністерство охорони здоров'я та соціального розвитку Російської Федерації, Науково-дослідний Інститут грипу (ФДБУ НДІ грипу). – СПб., 2010.
13. Юліш Є. І. Діти, що часто хворіють, і тактика педіатра / Юліш Є. І., С. Я. Ярошенко // Здоров'я дитини. – 2013. – Т. 49, №6 – С. 101–108.
14. Юліш Є. І. Ефективність імунофлазиду у профілактиці гострих вірусних інфекцій у дітей дошкільного віку / Юліш Є. І. // Сучасна педіатрія. – 2009. – Т. 26, №4. – С. 100–107.
15. Flavonoid-membrane interactions: involvement of flavonoid-metal complexes in raft signaling / Y. Tarahovsky [et al.] // Biochim. Biophys. Acta. – 2014. – Vol. 1838 (5). – P. 1235–1246.

Reference: O. V. Zubarenko, Yu. V. Desiatska, I. M. Shevchenko, N. Yu. Gornostaieva. *Immunoflazid® in the prevention of infectious diseases in infants // Modern Pediatrics. – 2015. – 3(67). – pp. 49–53.*

1.3. ACUTE RESPIRATORY INFECTIONS IN CHILDREN, THE LATEST TRENDS IN ANTIVIRAL THERAPY

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Abstract

The article is devoted to a topical issue in the practice of family doctors and pediatricians, namely acute respiratory viral diseases in young children and their modern etiotropic treatment. The study of the clinical effectiveness of the drug FLAVOVIR® in the treatment of acute respiratory viral infections in young children was conducted. Safety, tolerability of the drug and significant effectiveness allow recommending FLAVOVIR® for wide use in the treatment of acute respiratory viral infections in children, both in outpatient and inpatient settings.

Key words: acute respiratory viral diseases, children, treatment, FLAVOVIR.

Introduction

Today, the relevance of the issue of treatment and prevention of the acute respiratory infections (ARI) in children remains indisputable in the practice of the family doctors and pediatricians [1, 13]. ARI in children is the most common reason of calling a doctor and asking for a home visit or seeking outpatient care.

Annually, in Ukraine, 10–14 million people fall ill with the ARI accounting for 25–30% of the overall incidence and about 75–90% of the infection incidence [11]. Experts of the World Health Organization note that this most common group of diseases in the human population has demonstrated a constant increasing trend in the recent years. Social reasons associated with the global process of persistent urbanization, closer contacts between people almost anywhere in the world and increased intercontinental migration processes will contribute to the further spread of ARI.

Acute respiratory infections are the most common infectious diseases that affect all age groups on the planet, but the children population prevails. Viruses account for the largest portion in the etiological structure of ARI (70–75%) [1, 2]. Today, a significant number of pathogens of the acute respiratory viral infections (ARVI) are known; mainly they belong to 9 different groups of viruses – influenza, parainfluenza, adenovirus, respiratory syncytial virus (RSV), picornavirus, coronavirus, rheovirus, enterovirus and herpesviruses. Whereas, 16–23% of ARI cases are caused by bacteria (*Str. pneumoniae*, *Str. pyogenes*, *H. influenza type b*, *Moraxella catarrhalis*, *Staphylococcus spp.*, *gram-negative cocci*, etc.), approximately 1% is presented by the intracellular pathogens (*M. pneumonia*, *Chl. pneumonia*, *Chl. Trachomatis*, etc.), fungi (*Candida* and *Aspergillus genus*), protozoa (*Pn. carini*,

Pn. jieroveci) and their associations. According to the epidemiological data, ARVI accounts for 90% of cases in the infectious pathologies in children [1, 4].

According to WHO, on average, every child has 3 to 6 episodes of the ARVI per year. High ARVI prevalence is explained by the variability of pathogens and high contagiousness [1, 2, 8, 11].

Usually school-aged children have mild ARVI and in the young children these infections may cause such complications as otitis, sinusitis, bronchitis and pneumonia. To date, a number of risk factors associated with the common ARVI in children have been identified. One of them is so called “late start” – specific feature of immune system development in young children. It is known that early age is one of the critical stages in the child’s life. It is associated with the decreased maternal antibodies received by child during the prenatal development period. The anatomic and physiological characteristics of children are also the important risk factor for the common ARVI, as they make them more susceptible to infection. The most significant ones are the narrow respiratory tract, mucous membrane predisposition to swelling and increased secretion. The favorable cause of ARVI in children is the impact of the environmental factors (type of feeding, early socialization – early start of attending the preschool institutions, passive smoking, climatic conditions, etc.) [1, 2].

ARVI in children are similar by the epidemiological characteristics, pathogenetic mechanisms of development and clinical manifestations. The upper respiratory tract mucous membranes (nose, pharynx, larynx and trachea) are the site of entry for the pathogens of respiratory viral infections, where the active virus replication occurs. The initial viral entry into the epithelial cells triggers the local inflammatory response and leads to the clinical manifestations of catarrhal syndrome (nasal congestion, runny nose, lacrimation, cough, sore throat). Subsequently, the cellular debris is spread into the blood flow causing the systemic toxic manifestations, i.e.: intoxication syndrome (fever, impaired appetite or food refusal, fatigue, weakness, sleep disturbance). And virus presence in the body will support these pathophysiological processes. Intoxication symptoms are relieved only after the viral load is significantly decreased or virus is completely eliminated; usually it is achieved due to the normal activation of the child’s immune response. Often, especially in the young children, the insufficient immune function is observed and if the treatment is incorrect, the endogenous microflora is activated. It contributes to the development of complications (sinusitis, otitis, bronchitis, pneumonia) [1, 2, 8, 13].

A primary care provider has difficulties to verify the ARVI diagnosis, determine the specific pathogen, select the therapeutic approach, etiotropic therapy and prevent complications. For the purpose of the differential diagnosis of pathogens, the laboratory diagnostics is needed in addition to considering the clinical pathognomonic features of the disease. Today, the serological and virological methods are available to diagnose the viral infections [1, 2]. However, these methods of viral agent identification are often time-consuming, expensive and not always accessible for the broad routine medical practice. Considering the difficulties in verifying the ARVI etiological agent at the first days of disease onset, when choosing the etiotropic therapy a doctor pays maximum attention to the characteristics of the clinical picture of the disease. However, the clinical presentation has its own characteristic features in each case; commonly the combination of several pathogens is observed making it impossible to identify a specific pathogen based on the characteristics of the symptoms. Therefore, on the one hand, ARVI is the most common diagnosis, but on

the other hand, the etiotropic therapy should be able to have an effect on the non-specific ARVI pathogens.

ARVI prevention is still the relevant issue in medicine considering its seasonal occurrence, large-scale involvement and cyclicity among children. The most effective method of preventing infectious diseases is the active specific immunization. Recently, the specialists have been increasingly calling to use the medicinal products that nonspecifically affect immunity with caution and reasoning. However, parents, by themselves, often use various over-the-counter products available in the network of pharmacies intended to maintain the nonspecific protection from the infections, which can negatively affect the activity and efficiency of the immune system and contribute to the deterioration of the child's condition.

The heterogeneity of the respiratory infections does not allow a doctor to be limited by prescribing only the specific vaccines, but necessitates using the etiotropic products that should have maximum antiviral effects. Today, various groups of antiviral medicinal products with the direct mechanism of action are offered among the medications used for treatment and prevention of ARVI and influenza [4, 5, 12]. However, some of the medicinal products proposed at the pharmaceutical market have the narrow spectrum of action, age restrictions and important contraindications. Therefore, every day, a doctor encounters a difficult task to choose the medicinal product for ARVI treatment or prevention. Considering the etiopathogenetic aspects of ARVI and the routes of infection, it is possible to preliminarily distinguish the following pharmacodynamic components when prescribing antiviral medicinal products: direct antiviral action at all stages of viral infection development; broad-spectrum direct antiviral action with the effect on more than one type of pathogen; ability to inhibit the neuraminidase activity of influenza viruses; high bioavailability to the mucous membranes of the respiratory tract; antioxidant mechanism of action; safe immunotropic action without development of the refractory immune cells [3, 4, 5, 7, 8, 11, 13].

In the pediatric practice, closer attention has always been paid to the herbal products, as they have the high therapeutic efficacy and show more gentle and physiological interaction with a child's fragile body. Therefore, today the herbal products are actual and popular agents due to their certain positive properties. The latest innovative technologies allow obtaining medicinal products from the herbal raw materials that are safe and effective in the treatment of respiratory infections, especially in children.

Medicinal product FLAVOVIR® (SMC "Ecopharm" LTD) was registered at the pharmaceutical market of Ukraine long ago. The medicinal product is manufactured as syrup (30 ml and 50 ml) containing liquid extract PROTEFLAZID® derived from the herbal mixture (1:1) of *Herba Calamagrostis epigeios* L. and *Herba Deschampsia caespitosa* L. Many preclinical studies demonstrated the direct antiviral effect of the product involving inhibition of the virus-specific enzyme synthesis – DNA and RNA polymerases, thymidine kinase, reverse transcriptase, neuraminidase, 3CL-protease. Due to the direct effect on the ARVI cause (viruses), FLAVOVIR® syrup decreases the viral load in the child's body and allows coping with the infection more easily and without complications. In addition to the long-studied effect on the ARVI viruses, at the beginning of the new pandemic that swept the world, the Ukrainian and foreign scientists conducted a few studies of interaction of the active substance PROTEFLAZID® containing in FLAVOVIR® syrup with the new aggressor.

As a result, it was demonstrated that proteflazid flavonoids inhibited the activity of not only the RNA polymerase but also 3CL-protease of the pandemic coronavirus SARS-CoV-2 inhibiting the pathogen replication and ensuring the specific antiviral activity[6, 9, 10, 12].

Liquid form of FLAVOVIR® syrup ensures delivery of the active substance directly to the cells of the mucous membrane of the upper respiratory tract. The simple and understandable regimen of prescribing the syrup (only twice a day) and convenient dosing using a special dosing device make it popular among mothers. The presented medicinal product, in addition to the marked direct antiviral effect, also has the anti-inflammatory, detoxifying effect, antioxidant activity and ensures the adequate immune response to the pathogen. It may be used for a long time; when needed, it can be administered in combination with antibiotic. FLAVOVIR® does not have any age restrictions; it is used from birth.

The positive, effective pharmacodynamic properties of this syrup are the important alternative when choosing an antiviral agent for both the prevention and treatment of the present-day ARVI and influenza in children.

Study objective: investigate the clinical efficacy of the medicinal product FLAVOVIR® (syrup) used in treatment of the acute respiratory viral infections in the young children.

Study materials and methods

The study enrolled 60 young children receiving the outpatient treatment at the primary health care centers of Vinnytsia region from 2019 to 2021. The following inclusion criteria were used for children: presence of ARVI and age of 1 month to 3 years. Exclusion criteria involves children with the burdened premorbid background (immunodeficiency diseases, congenital malformations, etc.), use of other antiviral products, influenza vaccination.

Based on the objective, the children were assigned into two comparison groups. The first group included 30 children with the acute respiratory viral infection who received the medicinal product FLAVOVIR® (SMC “Ecopharm”, Ukraine) in combination with the symptomatic therapy. The second comparison group was composed of 30 patients with the acute respiratory viral infection who received only the symptomatic therapy. The patients were assigned to the groups by randomization. The groups were representative in terms of the age, gender, structure, and disease severity.

The clinical efficacy of FLAVOVIR® syrup was determined by comparison of the changes of clinical symptoms over time, disease duration and main laboratory blood parameters (complete blood count).

Medicinal product FLAVOVIR® was prescribed in the form of syrup to the young children by the following dosage regimen at the age-related doses: children under 1 year – 0.5 ml twice daily for 5 days; children 1 to 2 years – 1 ml twice daily for 5 days; children 2 to 3 years – 3 ml twice daily for 5 days. Statistical processing of the obtained study results was performed using standard software Statistica 5.0 for Windows XP.

Study results and their discussion

The clinical efficacy of medicinal product FLAVOVIR® used in children with ARVI was analyzed. Disease duration and severity of the clinical symptoms were evaluated. The clinical course of ARVI in the young children was characterized by the presence of the intoxication (increased body temperature from subfebrile to febrile, weakness, decreased appetite, drowsiness) and catarrhal (nasal congestion, rhinitis, hoarseness, lacrimation, cough) syndromes (Table 1).

Table 1. Clinical characteristics of ARVI in the examined young children

Clinical syndromes	Group I (FLAVOVIR®) n = 30		Group II n = 30	
	Abs.	%	Abs.	%
Intoxication				
fever	16	53,3	25	83,3
decreased appetite	12	40,0	6	20,0
sleep disturbance	6	20,0	8	26,7
fatigue	16	53,3	17	56,7
Catarrhal				
rhinitis	28	93,3	24	80,0
hoarseness	23	76,7	18	60,0
cough	18	60,0	21	70,0
lacrimation	11	36,7	8	26,7
Changes of peripheral blood parameters (leukopenia with lymphocytosis)	25	83,3	22	73,3

Young children who received FLAVOVIR® demonstrated shorter ARVI duration and generally mild to moderate clinical manifestations, while children from group II who did not receive FLAVOVIR® were much often diagnosed with the severe disease compared with group I.

Intoxication syndrome in children from study group I was characterized by the subfebrile temperature (53.3% of cases), moderate weakness and decreased appetite (40%). Intoxication symptoms in that group passed 3 times faster (group I: mean duration 3.4 ± 0.2 days; group II: 10.4 ± 0.6 days, $p < 0.05$) (Table 2). The following manifestations of the catarrhal syndrome were observed in children who received FLAVOVIR® syrup: nasal congestion, rhinitis (93.3%), lacrimation (36.7%), hoarseness (76.7%), infrequent dry cough (60%); mean duration of this syndrome was 4.5 ± 0.4 days.

Table 2. Evaluation of duration of clinical manifestations in young children with ARVI who received treatment

Clinical syndromes	Group I (FLAVOVIR®) n = 30	Group II n = 30	p
Intoxication	3.4 ± 0.2 days	10.4 ± 0.6 days	$p < 0.05$
Catarrhal	4.5 ± 0.4 days	9.6 ± 0.7 days	$p < 0.05$
Changes of peripheral blood parameters (leukopenia with lymphocytosis)	5.81 ± 0.5 days	11.8 ± 0.6 days	$p < 0.05$

At the same time, during the study, group II demonstrated more marked symptoms of intoxication (temperature from subfebrile (23.3%) to febrile (60%), decreased appetite up to complete food refusal in some subjects (20%), sleep disturbance, fatigue (56.7%)). Catarrhal syndrome in subjects from that group was also characterized by the rhinitis (80%), lacrimation (26.7%), cough (70%) and hoarseness (60%). Duration of catarrhal syndrome in group II was 9.6 ± 0.7 days (Table 2). Half of the children who

received only symptomatic therapy for ARVI treatment were diagnosed the different complications (otitis (10%), bronchitis (20%), pneumonia (30%)).

Follow-up results demonstrated that clinical manifestations of ARVI passed 2 times faster in the children from study group I compared with group II (group I: on average 5.81 ± 0.5 days, group II: 11.8 ± 0.6 days, $p < 0.05$).

To study the clinical efficacy of FLAVOVIR® syrup in children from both groups, monitoring of standard peripheral blood parameters was performed pre-treatment and over the time of follow-up. In particular, the following changes confirming presence of viral infection were observed in the vast majority of children from group I (83.3%) and group II (73.3%): leukopenia with lymphocytosis. The hemogram parameters were normalized on day 5–6 of the disease secondary to FLAVOVIR® syrup dosing, while in children from group II – only following 10–14 days.

It should be noted that analysis of safety of antiviral agent FLAVOVIR® used in ARVI treatment in the young children demonstrated that neither parents nor we observed any side effects in all children from group I.

Conclusions

In the present-day conditions of medicine development, it is possible to use the effective and safe methods to treat the acute respiratory viral infections in children. During the conducted study, the medicinal product FLAVOVIR® demonstrated the efficacious antiviral effect in ARVI treatment in the young children. FLAVOVIR® syrup addition to the dosage regimen of the subject showed the positive changes of the disease clinical course: intoxication syndrome resolved 3 times faster (group I: mean duration 3.4 ± 0.2 days; group II: 10.4 ± 0.6 days, $p < 0.05$), catarrhal syndrome – approximately 2 times (group I: mean duration 4.5 ± 0.4 days; group II: 9.6 ± 0.7 days, $p < 0.05$); period of recovery was about 2 times shorter (group I: mean duration 5.81 ± 0.5 days; group II: 11.8 ± 0.6 days, $p < 0.05$). The medicinal product was well tolerated by the patients and is safe for children.

The obtained results allow recommending medicinal product FLAVOVIR® (syrup) at the age-related doses in the etiotropic therapy of the present-day acute respiratory viral diseases in the young children taking into account the direct antiviral effect of the medication.

References:

1. Brealey J. C., Sly P. D., Young P. R., Chappell K. J. Viral bacterial co-infection of the respiratory tract during early childhood. *FEMS Microbiol Lett.* 2015 May;362(10):fnv062. doi: 10.1093/femsle/fnv062. Epub 2015 Apr 15. PMID: 25877546.
2. Heinonen S., Rodriguez-Fernandez R., Diaz A., Oliva Rodriguez-Pastor S., Ramilo O., Mejias A. Infant Immune Response to Respiratory Viral Infections. *Immunol Allergy Clin North Am.* 2019 Aug; 39(3):361–376. doi: 10.1016/j.jac.2019.03.005. Epub 2019 May 15. PMID: 31284926; PMCID: PMC6625527.
3. Kai H., Obuchi M., Yoshida H. et al. (2014) *In vitro* and *in vivo* anti-influenza virus activities of flavonoids and related compounds as components of Brazilian propolis (AF-08). *J. Funct. Foods*, 8: 214–223.
4. Liu A. L., Wang H. D., Lee S. M. et al. (2008) Structure-activity relationship of flavonoids as influenza virus neuraminidase inhibitors and their *in vitro* anti-viral activities. *Bioorg. Med. Chem.*, 16(15): 7141–7147.
5. Lu S. J., Chong F. C. (2012) Combining molecular docking and molecular dynamics to predict the binding modes of flavonoid derivatives with the neuraminidase of the 2009 H1N1 influenza A virus. *Int. J. Mol. Sci.*, 13(4): 4496–4507.

6. PubMed//Proteflazid® effectiveness for prevention and treatment of acute viral respiratory infections in the conditions of COVID-19//pubmed.ncbi.nlm.nih.gov/34464364//2021
7. Tarahovsky Y. Flavonoid-membrane interactions: involvement of flavonoid-metal complexes in raft signaling / Y. Tarahovsky [et al.] // Biochim Biophys Acta. – 2014. – Vol. 1838 (5). – P. 1235–1246.
8. Бекетова Г. В., Хайтович М. В., Гриневич О. Й. (2014) Імунофлазід у педіатрії: системний аналіз ефективності та безпеки застосування // Педіатрія. Східна Європа, 3 (7): 141-152.
9. Інструкція для медичного застосування препарату ФЛАВОВІР® зі змінами №1922 від 10.09.2021.
10. Інформаційний лист про нововведення у сфері охорони здоров'я. Напрямок впровадження: віруси та вірусні інфекції. Антивірусна активність флавоноїдного препарату Протефлазід® // Укрмедпатентінформ, №80. – 2021. – 4 ст.
11. Крамарьов С. О., Гриневич О. Й., Тонковід О. Б., Виговська О. В. (2014) Метааналіз результатів клінічних досліджень ефективності флавоноїдів при вірусних та вірусно-бактеріальних захворюваннях у дітей // Сучасна педіатр., 5 (61): 1–7.
12. Рибалко С. Л., Старосила Д. Б., Завелевич М. П. Сучасний стан хіміотерапії та профілактики грипу та ГРВІ в Україні // Укр. Мед. Часопис, 1 (123), Т.1 – І/ІІ 2018 р.
13. Токарчук Н. І. Використання імунофлазиду для профілактики та лікування грипу та ГРВІ у дітей під час сезонного підвищення захворюваності // Сучасна педіатрія. – 2012. – №1 (41). – С. 123–127.

1.4. THE EFFICACY OF THE PREVENTIVE USAGE OF FLAVONOIDS AMONG THE SCHOOL AGE CHILDREN IN THE COMPLEX REDUCTION OF THE ACUTE RESPIRATORY DISEASES INCIDENCE

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Abstract

Objective: to study the clinical and immunological efficacy of flavonoids use (FLAVOVIR®, PROTEFLAZID®) with preventive purpose in school age children in a complex of measures to reduce the incidence of acute respiratory diseases. Materials and methods. A total of 78 children aged 7 to 17 years, for the prevention of acute respiratory disease were assigned PROTEFLAZID® (children up to the age of 9 used the FLAVOVIR® syrup, and children older than 9 were administrated PROTEFLAZID®, in a dose corresponding to the manufacturer's medicinal product label) in the season of different calendar years. The duration of seasonal prevention of ARI for all children was 2 weeks. Clinical efficacy of the flavonoids entry in the seasonal prevention regimen was assessed by the intensity and course of acute respiratory disease, the dynamics of the mucocutaneous biocenosis of the upper respiratory tract. The immunological examination (leukocytes, lymphocytes, CD25+, IgA, IgM, IgG, TF1 (DRG, ELISA, Germany), IL-4, IL-10, IL-12 p70 and IL-12 p40+p70 (ELISA, Diaclone, France), IL-2 (ELISA Kit, Finland) in serum was used for appraisal as well. Statistical analysis of the research results was carried out by using of the statistical programs Statgraf, Matstat, Microsoft Excel for Windows, and by applying para and nonpara metric reliability criteria. Results. Carrying out the preventive measures in children of school age using PROTEFLAZID® (FLAVOVIR®, PROTEFLAZID®) makes it possible to reduce the incidence of acute respiratory infections by 1.2–1.5 times, and in case of disease, reduce the duration of an acute respiratory episode by 1.8–2.2 days. In addition, all emerging acute respiratory infections had low grade severity. The efficacy of ARI preventive prophylaxis in children aged 7–17 years with FLAVOVIR® syrup or PROTEFLAZID® drops made up in average 82%, was characterized as well tolerated, and provided the formation of eubiosis of the upper respiratory tract mucous membranes in 64.1% of cases. One of the main immunological effects of PROTEFLAZID® (FLAVOVIR®, PROTEFLAZID®) is its preventive control the possibility of inflammatory process prolonging at the immunological level in school age children. Conclusions: the use of PROTEFLAZID® in the prophylactic regimens of seasonal ARI in school age children is effective and appropriate.

Key words: acute respiratory diseases, children, prophylaxis, PROTEFLAZID®.

Introduction

Today, the primary care providers and pediatricians have a lot of medicinal products and dosage regimens intended to prevent the acute respiratory diseases (ARD). The

medicinal product is commonly chosen based on the data of its efficacy and safety, particularly considering the expected effect of the therapy on the condition of the child's immune system [4, 8, 9]. One of the best methods of ARD prevention is the immune system modulation intended to achieve the balanced function the patient's immune system by using the multidirectional effects of the medication components [1, 8, 9]; it is manifested in the clinical settings as prevention of the primary manifestations of the clinical signs or disease recurrence [2, 3, 6, 10, 11].

The choice of the medicinal product and its dosage regimen for ARD prevention in children is still a challenge, as the list of the active substances and medicinal products that may be possibly used to prevent ARD is quite big and is constantly increasing, but the choice of a medical practitioner should be grounded on the evidence-based medicine [2, 6, 7].

The analytical review of the literature performed by us and existing evidence base of the studies of efficacy of bioflavonoids of the natural origin, i.e. active substance PROTEFLAZID® (medicinal products FLAVOVIR® (syrup) and PROTEFLAZID® (drops)) [1–4, 6–11] allow indicating the etiologically and pathogenetically justified prescription of these bioflavonoids in both treatment and prevention regimens of ARD due to their direct antiviral effect and antioxidant activity in combination with the immuno-corrective properties, as well as proven high efficacy and safety of use in the viral and viral-bacterial diseases in children.

Study objective was to investigate the clinical and immunologic efficacy of the preventive use of bioflavonoids (FLAVOVIR®, PROTEFLAZID®) in the school-aged children in combination with the measures intended for reduction of ARD morbidity.

Materials and methods

Seventy eight (78) children aged 7 to 17 years (mean age: 12.1 ± 0.3 years) were followed-up; there were 44 (56.4%) boys and 34 (43.6%) girls among them. All children received medicinal product PROTEFLAZID® (FLAVOVIR®, PROTEFLAZID®) for ARD prevention during the season over the different calendar years – 38 children (mean age: 12.2 ± 0.4 years) received the preventive therapy at one of the autumn seasons and 40 children (12.1 ± 0.5 years; $p > 0.90$ between seasons) received it at another season; their data were similar allowing combining the results to make the final conclusions.

Based on the objective, 68 of those 78 children received one-time course of preventive therapy and 10 subjects with the recurrent ARD received the course of preventive therapy twice with 12-month interval.

Children under 9 years old were prescribed FLAVOVIR® syrup and children aged 9 years and older received PROTEFLAZID® (drops) in accordance with the age-related doses and manufacturer instruction. Duration of the ARD seasonal prevention was two weeks for all children.

The clinical efficacy of adding the flavonoids to the seasonal prevention regimen was assessed by the ARD intensity (results of one- or two-year follow-up after the preventive measures were completed), changes of the biocenosis of the mucous membranes of the upper respiratory tract (URT), evaluation of the clinical characteristics of ARD course in the follow-up and results of the immune examination over time of follow-up.

All followed-up children had the following examinations: review of the anamnestic data, assessment of the physical examination data prior to and after the preventive therapy course, follow-up of the children after they completed the preventive course with the medicinal product PROTEFLAZID® (drops); microbiological test for the colonization of the pharyngeal and nasal mucosa over the period of treatment performed twice: prior to and after

the preventive therapy with the medicinal product PROTEFLAZID®; the immune status was evaluated by the count of white blood cells, lymphocytes, CD25+, IgA, IgM, IgG, TF-1 (DRG ELISA, Germany) level, content of serum IL-4, IL-10, IL-12 p70 and IL-12 p40+p70 (ELISA, Diaclone, France), IL-2 (ELISA Kit, Finland) cytokines prior to and after the preventive therapy course with PROTEFLAZID®.

The mathematical processing of the study results was performed using Statgraf, Matstat, Microsoft Excel for Windows statistical programs; both parametric and non-parametric tests of significance were used to analyze the significance of the statistical samples [5].

Study results and their discussion

The follow-up of all children who were observed within a year after enrollment to the seasonal prevention with medicinal product PROTEFLAZID® (FLAVOVIR®, PROTEFLAZID®) allowed to determine 82% mean efficacy of the preventive prophylaxis with bioflavonoids in children due to the decreased ARD frequency by 1.2–1.5 times and reduced duration of the acute respiratory episode 1.8 to 2.2 days (Fig. 1).

In addition to the decreased ARD incidence in the school-aged children, the number of cases of the complicated ARD course was reduced by 30%. Also, no moderate or severe disease was observed in every second child who received the preventive course of PROTEFLAZID® (FLAVOVIR®, PROTEFLAZID®) at the start of the season but after some time, still fell ill with ARD due to the high acute respiratory morbidity in the population (Fig. 1).

It should be noted that the children with the recurrent ARD (10 [12.8%] subjects) who received prevention with the medicinal product PROTEFLAZID® (FLAVOVIR®, PROTEFLAZID®) twice with 12-month interval over one-year follow-up after each of preventive course using those bioflavonoids demonstrated the ARD incidence reduced to the level of children who fall ill episodically meeting the incidence criteria of almost healthy child (only 1–2 episodes of ARD during the follow-up). Two among those 10 children did not fall ill at all within a year.

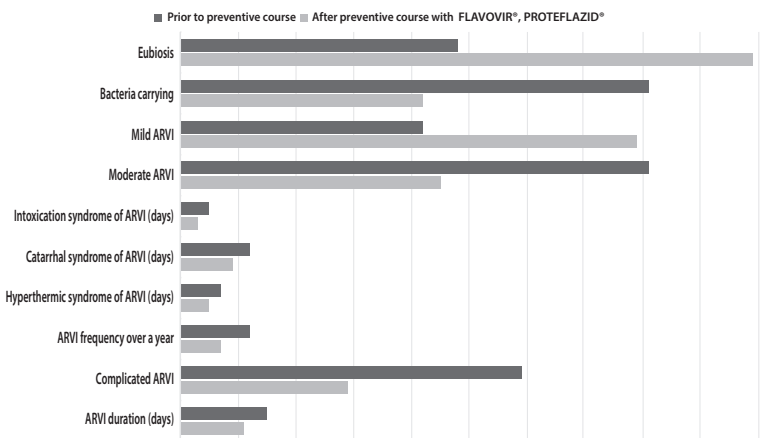


Figure 1. Clinical efficacy of preventive course with bioflavonoids (FLAVOVIR®, PROTEFLAZID®) in the prophylactic regimen of seasonal ARD in the school-aged children

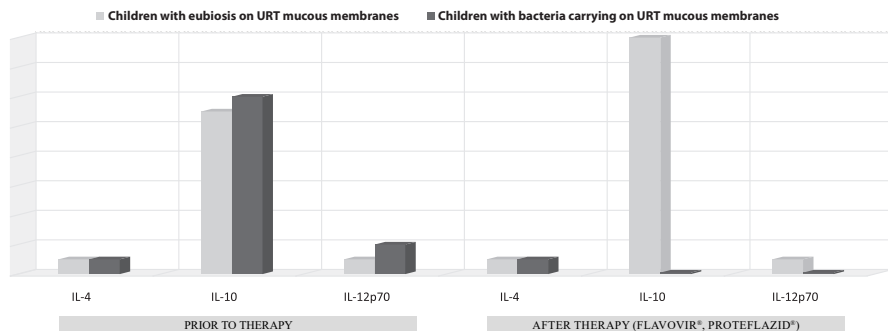


Figure 2. Immunological efficacy of therapy with bioflavonoids (FLAVOVIR®, PROTEFLAZID®) in the school-aged children depending on colonization of URT mucous membranes

Examination of the pharyngeal and nasal mucosa biocenosis in all school-aged children at the clinical well-being condition just prior to the prevention with bioflavonoids (FLAVOVIR®, PROTEFLAZID®) showed that only 24 (30.8%) children had the normal inoculated flora corresponding to the eubiosis of the upper respiratory tract; the rest children demonstrated the presence of the opportunistic and pathogenic flora on the URT mucosae. Their bacteriological verification showed that the recovery of *St. aureus* (60.4%) was in the first place and *Str. haemolyticus* (35.4%) – in second place.

Control microbiological tests for the URT mucosa biocenosis (Fig. 1) in children immediately after completion of the preventive course with bioflavonoids (FLAVOVIR®, PROTEFLAZID®) showed that eubiosis was formed in most cases – 64.1% (n = 50) – versus 30.8% (n = 24) prior to the preventive therapy course ($p < 0.001$).

Our previous studies of the impact of the impaired URT microbiocenosis on the immune status in children who were bacteria carriers as the presence of the prolonged low-level inflammatory Th¹-associated response were the reason to perform the comparative analysis of the immunological efficacy of the bioflavonoids effect in the followed-up children depending on the mucosa colonization (Fig. 2).

Based on the results of this study, after the preventive course with bioflavonoids (FLAVOVIR®, PROTEFLAZID®) (Fig. 2) was completed, the immunological changes were recorded in the children who were bacteria carriers due to the significantly increased serum IL-10 ($t = 2.04$; $p < 0.05$) and decreased serum IL-12 p70 content ($t = 3.5$; $p < 0.01$) allowing to indicate that the bioflavonoids had the modulating effect due to the regulated activity of pro- and anti-inflammatory interleukins ($p < 0.01$).

According to our data, the immune status of the school-age children during the period of clinical well-being and prior to the initiation of the preventive measures to reduce the seasonal ARD was characterized by the non-persistent manifestations of the insufficient components of the immune system that are quite variable and first of all demonstrated the imbalanced levels of regulation and stressed immune defense mechanisms.

Therefore, the evaluation of the immunological efficacy of adding bioflavonoids (FLAVOVIR®, PROTEFLAZID®) to the ARD preventive prophylaxis in the school-aged children was performed

in groups differed by the trend of the pro- and anti-inflammatory interleukins, i.e.: in 35 school-aged children with the relative high initial level of IL-12 p70 (first group of follow-up) and 43 children with the relative low initial level of IL-12 p70 (second group of follow-up). Based on the obtained results (Fig. 3), adding bioflavonoids (FLAVOVIR®, PROTEFLAZID®) to the ARD seasonal prevention in children from the first group of follow-up resulted in the significantly decreased IL-12 p70 content ($t = 4.03$; $p < 0.001$) secondary to the significantly increased pro-inflammatory IL-10 ($t = 2.3$; $p < 0.05$) level, while serum IL-4 content in those children almost did not change ($p > 0.05$). In children from the second group of follow-up (Fig. 3), effect of the bioflavonoids was associated with the significantly increased IL-12 p70 content ($t = 2.23$; $p < 0.05$), while serum IL-4 and IL-10 levels did not change ($p > 0.05$)

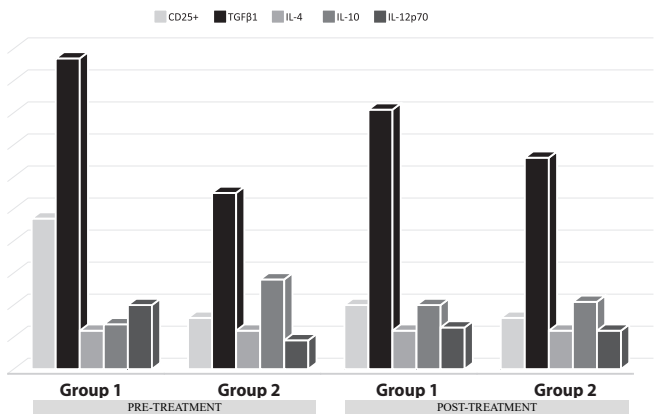


Figure 3. Changes of immunological parameters in the school-aged children based on the cytokine balance secondary to the correction with bioflavonoids (FLAVOVIR®, PROTEFLAZID®)

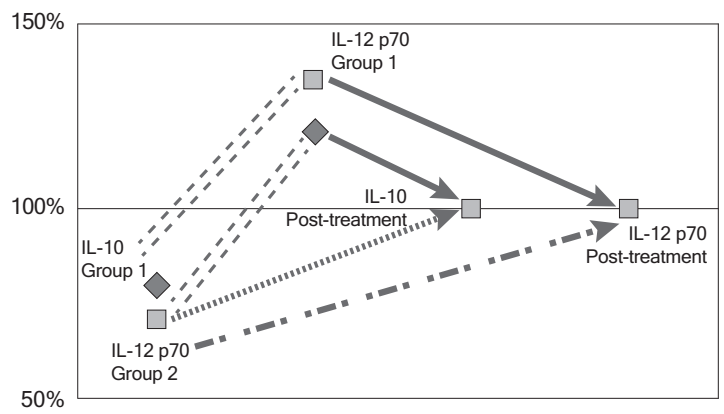


Figure 4. Effect of bioflavonoids (FLAVOVIR®, PROTEFLAZID®) on the balance of pro- and anti-inflammatory cytokines in groups of children based on the initial serum IL-12 p70 level

Comparison of the results of immune examination in the school-aged children who completed the preventive course with bioflavonoids (FLAVOVIR®, PROTEFLAZID®) (Fig. 3) did not detect the significant differences between the serum IL-4, IL-10 and IL-12 p70 interleukin content in the children from both groups ($p > 0.05$) suggesting the same pattern of cytokine distribution as the resulting characteristic of the modulating action of flavonoids (graphically presented in Fig. 4). Effect of FLAVOVIR® syrup and PROTEFLAZID® drops on the IL-12 p70 content is mediated by the modulation of IL-10 level in such a way that at the high initial level of pro-inflammatory IL-12 p70, the bioflavonoids inhibit and at the low level they activate the production of the anti-inflammatory IL-10, which, in our opinion and taking into consideration the theoretical studies of the molecular effects of cytokine regulation, most probably balances Th¹- and Th²-response in the school-aged children due to the regulation of activity of STAT-3 transcription factors causing the normalization of the serum IL-12 p70 levels after immune correction with bioflavonoids is completed.

The medicinal product PROTEFLAZID® (FLAVOVIR®, PROTEFLAZID®), regardless of the initial serum IL-12 p70 level modulates its serum concentration: if its pre-correction level was high, it decreases it up to the mean one, if the level was low, then on the contrary it increases post correction. Considering that pro-inflammatory IL-12 p70 supports inflammation development in the body, it is possible to tell that one of the main immunologic effects of the medicinal product PROTEFLAZID® (FLAVOVIR®, PROTEFLAZID®) is its ability to preventively control the prolongation of the inflammatory process at the immunological level in the school-aged children.

According to our data, all children who received the medicinal product PROTEFLAZID® (FLAVOVIR®, PROTEFLAZID®) tolerated its administration well; no allergic or any side effects were observed in any child.

Therefore, the addition of the naturally synthesized bioflavonoids (FLAVOVIR®, PROTEFLAZID®) to the ARD preventive seasonal prophylaxis in the school-aged children is the safe and effective measure confirmed by the proven effect on immune status parameters, accompanied by the restoration of the micro-ecology of the URT mucosae and has the clinically proved efficacy. It allows recommending using the medicinal products FLAVOVIR® (syrup) and PROTEFLAZID® (drops) in the seasonal preventive regimens of the acute respiratory morbidity.

Conclusions

1. The school-aged children in the clinical well-being period do not have the persistent manifestations of the insufficient immune system components; they are rather variable indicating the imbalanced levels of regulation and stressed immune defense mechanisms.

2. Bacteria carrying (due to *St. aureus* and *Str. haemolyticus* inoculated on the URT mucosae) was recorded in 69.2% of the school-aged children in the period of the somatic health.

3. Preventive measures taken in the school-aged children and using the medicinal products FLAVOVIR® (syrup) and PROTEFLAZID® (drops) allow decreasing the ARD incidence by 1.2–1.5 times and in case of falling ill – reducing the duration of the acute respiratory episode by 1.8–2.2 days. Occurring ARD are characterized by the milder course (without moderate or severe disease).

4. Preventive use of the bioflavonoids (FLAVOVIR®, PROTEFLAZID®) results in the formation of URT mucosa eubiosis in 64.1% of cases.

5. One of the main immunologic effects of the medicinal products FLAVOVIR® (syrup) and PROTEFLAZID® (drops) is the ability to preventively control the possible prolongation of the inflammatory process at the immunological level in the school-aged children.

6. The mean efficacy of the ARD preventive prophylaxis in children aged 7–17 years using syrup is 82% and is characterized by the good tolerability indicating the high safety of these medicinal products.

Prospects for further studies. Considering our data and information from the literature pertinent to the components of bioflavonoids (FLAVOVIR®, PROTEFLAZID®) effect on the human body, further studies of the adaptive properties of these compounds at the different levels of the body's adaptation is promising and justified.

References:

1. Абатуров О. Є. Реалізація противірусної та антиоксидантної дії біофлавоноїдів при лікуванні гострих респіраторних вірусних інфекцій / О. Є. Абатуров, І. Л. Височина // Здоров'я дитини. – 2016. – С.128–134.
2. Бекетова Г. В. Імунофлазид® у педіатрії: системний аналіз ефективності та безпеки застосування / Г. В. Бекетова, Н. В. Хайтович, О. Й. Гриневич // Педіатрія. Східна Європа. – 2014. – №3 (07). – С.10.
3. Імунофлазид® сироп: лікування та профілактика ГРВІ та грипу // Новини медицини та фармації. – 2014. – №21–22 (523–524). – С.8–9.
4. Інструкція для медичного застосування препарату ФЛАВОВІР®. Затверджено наказом МОЗ України. Реєстраційне посвідчення № UA/5510/01/01 від 21.10.11 №685. Зміни внесені. Наказ Міністерства охорони здоров'я України від 06.10.2014 №705.
5. Лапач С. М. Статистичні методи у медико-біологічних дослідженнях з використанням Excel / С. М. Лапач, А. В. Чубенко, П. М. Бабич. – Київ: Моріон, 2000. – С.320.
6. Метааналіз результатів клінічних досліджень ефективності флавоноїдів при вірусних та вірусно-бактеріальних захворюваннях у дітей / С. О. Крамарьов, О. Й. Гриневич, О. Б. Тонковід, О. В. Виговська // Сучасна педіатрія. – 2014. – №5 (61). – С.1–7.
7. Моніторинг клінічного застосування лікарського препарату ПРОТЕФЛАЗІД краплі лікарями різних спеціальностей на території України у 2015 р. // Новини медицини та фармації. – 2016. – №14 (594). – С.5.
8. Рибалко С. Л. Вивчення механізмів дії біологічно активних речовин лікувальної субстанції ПРОТЕФЛАЗІД: звіт / С. Л. Рибалко – 2010 – С.84.
9. Сокурєнко Л. М. Антиоксидантна дія імунофлазиду при грипі / Л. М. Сокурєнко // Таврійський медико-біологічний. вісн. – 2012. – Т.15, №4 (60). – С.339–341.
10. Токарчук Н. І. Досвід використання ФЛАВОВІРУ в комплексі лікувально-профілактичних заходів під час сезонного підвищення захворюваності на грип та ГРВІ / Н. І. Токарчук, Л. С. Старинець // Перинатологія та педіатрія. – 2012. – Т.49, №1. – С.1–5.
11. Юліш Є. І. Діти, що часто хворіють, і тактика педіатра / Юліш Є. І., С. Я. Ярошенко // Здоров'я дитини. – 2013. – Т.49, №6. – С.101–108.

Reference: I. L. Vysochyna, O. Ye. Abatur. The efficacy of the preventive usage of flavonoids among the school age children in the complex reduction of the acute respiratory diseases incidence // Modern Pediatrics. – 2017. – 4(84). – pp. 55–60.

1.5. PREVENTIVE AND THERAPEUTIC EFFECTIVENESS OF BIOFLAVONOIDS IN CHILDREN WITH RECURRENT RESPIRATORY INFECTIONS (ARRI)

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Abstract

The aim of the study was to increase the effectiveness of treatment and prevention of acute recurrent respiratory infections (ARRI) by FLAVOVIR® syrup usage in children of primary school age.

Materials and methods. The study included 50 children of primary school age with ARRI. Preventive and curative efficacy of FLAVOVIR® syrup was determined by studying the dynamics of clinical symptoms within the year, parameters of saliva microcrystallization (SMC), bacterial composition of pharyngeal exudates. **Conclusions.** FLAVOVIR® syrup used for preventive and curative purpose improves general well-being in children, reduces the manifestations of toxipathy, asthenia, the frequency and severity of acute respiratory infections, number of sick days, the number and duration of hospitalizations, increases mineralizing potential of saliva against the background of reduction of the microbiological load of the oral cavity.

Key words: FLAVOVIR®; bioflavonoids; acute recurrent respiratory infections; treatment; prevention; schoolchildren.

Introduction

Analysis of the health status of the child population of Ukraine proves the existing trend towards the steadily increased proportion of the school-aged children who recurrently and for a long time suffer from the acute respiratory infections (ARI) [6, 7]. Children with the common upper respiratory tract diseases – acute recurrent respiratory infections (ARRI) – are a special problem for the pediatricians and family doctors. They account for majority of the pediatric visits, have a high rate of absence from school and hospitalizations; they are prescribed a wide range of the medications; the absence of the effective and timely preventive measures taken in this category of children leads to the chronic diseases developed in 70.0% of these high school students with the leading ENT disorders and bronchopulmonary diseases [13]. This group of children is quite diverse and heterogeneous. It is necessary to differentiate the children with the episodes of the recurrent viral infections and with the chronic diseases of ENT, respiratory tract, abnormal development of the respiratory tract, congenital or acquired immune dysfunctions, etc.

It is necessary to consider the fact that today the definition of the “recurrent respiratory infection” (RRI) is still disputable, arbitrary and too general by nature, without definite medical consensus. To diagnose RRI in children we followed the guidelines of the immunological group of the Italian Society of Pediatrics determining the presence of one of the following criteria [13]:

- ≥ 6 respiratory infections per year;
- ≥ 1 respiratory infection involving upper respiratory tract per month in the period between September and April;
- ≥ 3 respiratory infections involving lower respiratory tract per year.

Results of the conducted to date studies indicate that though the immune system of the children with ARRI does not have the major primary and acquired defects, it is characterized by the extremely intensified processes of the immune response, impaired intercellular cooperation and insufficient reserve capabilities that are the result of the long-term and substantial antigenic effect on the child's body [9–11].

Conducted by us studies demonstrate that majority of children with ARRI do not have the serious health problems, immune or non-immune pathology allowing determining that they have physiological condition of the respiratory system. It means that a certain number of them can be considered as the adaptive response of the child's body to the effects of the excessive load of infectious antigens, adverse environmental factors and socio-ecological problems. Partially decreased specific immune parameters detected in some children with ARRI were temporary and basically reflected the increased predisposition to the response to infectious agent which may confirm their secondary, post-infectious nature. Non-specific changes of the immune parameters are the consequence of the recurrent invasions by the infectious agents but not the favorable factor leading to their development. According to our data, the latent and recurrent intracellular infections (type 1 and 2 herpes virus, Epstein-Barr, cytomegalovirus, chlamydia and mycoplasma) were detected in 83.3% of children with ARRI significantly complicating the course of the inflammatory process and decreasing the efficacy of its treatment [6].

The problem of ARRI prevention and treatment is especially relevant in the period of formation of the school communities which are rightly classified as the high-risk groups since they are distinguished by the possibility of cross-infection due to the active mixing and are characterized by the increased level of morbidity that can also serve as the indirect confirmation of the immune status changes during this adaptation period [7]. The influenza vaccination is the indisputable effective way of prevention, but due to the constant change of the antigenic properties of the pathogen, the continuous monitoring and development of new vaccine strains circulating in the human population in each specific epidemic season are required.

The properties of FLAVOVR® syrup have been investigated in the experimental and clinical studies over the last few years. The active substance of the syrup is bioflavonoids (BF) of the wild *Calamagrotis epigeios* and *Deschampsia caespitosa* grasses – stable molecular combinations of the flavonoid aglycons composed of the free aglycons (tricine, apigenin, luteolin, quercetin and rhamnazin), O-glycosides and C-glycosides [1, 2]. The main characteristic of these bioflavonoids is their direct antiviral effect achieved due to their biological properties; in addition, they function as the antioxidants in the human body [1, 2, 8].

The flavonoids also exert the anti-inflammatory, antibiotic-like potential: acting as the antimicrobial agents, they inhibit the functioning of pathogenic microorganisms [12, 15].

It has been proved that the antioxidant activity of BF contained in FLAVOVR® syrup is achieved by inhibiting the generation of the activated oxygen-containing metabolites (AOCM) by the microorganism cells. The generation rate of the superoxide anion radical after 2-hour cell incubation with BF was decreased by 20–30%, after 4-hour incubation – by more than 50%, and after 24-hour incubation, AOCM generation was practically inhibited by the microorganism cells [8]. The flavonoids have an effect on the antioxidant system: at the stage of free radicals and peroxides formation they act as the free radical scavengers, inhibit some enzymes, provoke the oxidative process and protect the antioxidant enzymes from damage [16].

BF potentiate the effect of apoptosis inducers, activate the initiator caspase-9 by intracellular mechanisms and contribute to the elimination of affected cells, thereby achieving an apoptosis-modulating effect which is important for prevention of the chronic processes associated with the persistent latent viral infections [8].

In recent years, the effect of BF on the cellular receptor system, in particular on receptors that stimulate the detoxification processes, has been determined [17]. These properties are especially important for the children with ARRI, as the persistent inflammatory processes accompanied by the intensified production of pro-inflammatory cytokines and immune imbalance lead to the chronic intoxication and decrease of the adaptive properties of the body [9].

To prevent the polymedication in this category of children, it is important to reasonably use the medicinal products with the combined effect, which, in addition to the specific antiviral action, would have a wide range of immuno-modulatory and general health-improving properties resulting in the search of the new agents for prevention and therapy of the respiratory infections and determining the relevance of the study.

Objective

To increase the efficacy of treatment and prevention of the acute recurrent respiratory infections by using FLAVOVIR® syrup in children of elementary-school age.

Materials and methods

To fulfill the objectives, we examined 50 children aged 7–10 years with the recurrent respiratory infections that had more than six cases of ARI during the school year and, on average, had 35–40-day absence from school due to illness within the school year. The examinations and treatment in this group of children was agreed with their parents.

Followed-up children did not have clinically significant structural respiratory diseases and ENT disorders, congenital or acquired immune deficiencies. All children were followed-up for 12 months; they received the preventive (anti-relapse) courses using FLAVOVIR® syrup at the age-related doses for 3 weeks twice per year (September to October and February to March). Additionally, if the clinical symptoms of the respiratory disease were present, this group received the 5-day therapeutic course with the above mentioned medicinal product (not more than twice per year).

Efficacy of FLAVOVIR® syrup was determined by comparison of clinical symptom changes within a year, characteristics of the saliva microcrystallization (SMC), bacterial composition of the pharyngeal exudate prior to the study and after one-year preventive and treatment courses.

Examination of the crystal-forming function of the mixed saliva was performed by P. A. Leus method [4]. Dried saliva drops were examined under the microscope. Three types of the saliva microcrystallization were distinguished based on the crystal location: – type I SMC is characterized by the large tree-like and crystal-like structures located at the center (fern); the relative incidence of oral cavity and tonsil diseases in children with this type does not exceed 12–15%; – type II SMC is characterized by the isolated crystal-like conglomerates or needle-like crystals located throughout the field of vision; the relative incidence of oral cavity and tonsil diseases in children with this type does not exceed 25–30%; – type III SMC is characterized by the scattered single small crystals without a specific orientation (sometimes in the form of drops); this type is accompanied by the multiple uncompensated inflammatory oropharyngeal processes.

The results of the comparative assessment of microcrystallization parameters of three biological fluids (blood serum, oral fluid, urine) of the experimental animals and humans prove that saliva microcrystallization is a general indicator of the body’s homeostasis, which can be used both to evaluate the efficacy of the preventive, therapeutic and rehabilitation measures and to predict the course of diseases [3]. Some articles are devoted to the study of saliva microcrystallization in the different somatic pathologies [5, 14].

The study results were statistically processed using Excel software. The main characteristics are provided as the quantitative observations (n), arithmetic mean (M), standard error of the mean (m), absolute and relative values (abs.,%), level of statistical significance (p). Comparison of the statistical characteristics in different groups and over the time of follow-up was performed using parametric and non-parametric criteria. The t-test for independent samples was used to determine the statistical significance of differences between the independent groups while comparing the mean values of the studied variables.

Results

Study results indicated that after 12-month follow-up, the manifestations of the prolonged asthenic and intoxication syndromes were decreased showing increased physical activity and performance efficiency in 25 children (50.0%), decreased fatigue in 33 (66.0%), prolonged sleep duration in 16 (32.0%) and improved appetite in 26 (52.0%) ($p \leq 0.05$).

Decreased number of ARI episodes during the follow-up compared with the previous year was recorded in 16 (32.0%) children demonstrating the high efficacy of the received preventive courses (Table 1). One-two episodes of the respiratory disease were observed in 6 (12.0%) children. Follow-up of those children showed that their ARI was uncomplicated with the decreased duration and severity of the basic symptoms without the need for the additional medicinal products or inpatient treatment. Hemoglobin and WBC concentrations were normalized in 42.0% of children; their blood rheology parameters were improved.

The clinical efficacy of the preventive therapy was considered to be low in 13 (26.0%) children. Analysis of this group of children did not demonstrate any specific features of respiratory infection courses pertinent to their severity, complications or severe concomitant conditions; however, those children were from the large families with the low socio-economic status.

Table 1. Changes of specific clinical and laboratory parameters in children with ARRI (n=50)

Clinical symptom	Abs.	%
Increased physical activity and performance efficiency	25	5.0
Decreased fatigue	33	66.0
Prolonged sleep duration	16	32.0
Improved appetite	26	52.0
Normalization of hemoglobin level	21	42.0
Decreased number of ARI episodes	16	32.0
Decreased severity of ARI course and duration of basic symptoms	22	44.0

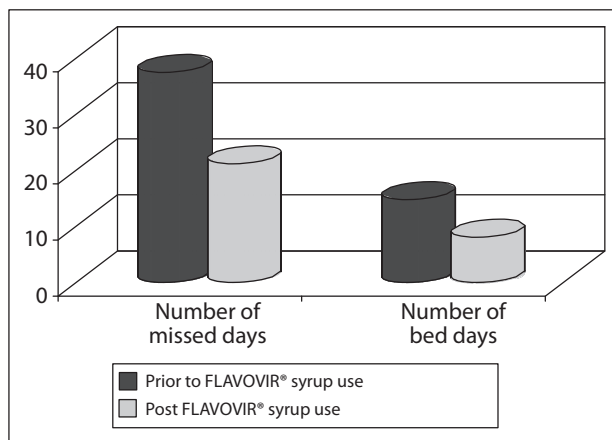


Figure 1. Number of missed days at school and bed days over the time of follow-up

Children who received FLAVO VIR® syrup for prevention and treatment purpose within a year demonstrated 3-fold decrease of the herpes virus infection reactivation rate; hospitalization rate was decreased by 19.0% and its duration – by 27.0%.

The assessment of number of days missed at school determined that the children missed 37.5 ± 3.7 days in the previous year and at the end of the follow-up – 21.2 ± 1.5 days ($p \leq 0.05$). The number of bed days spent in the hospital due to the complicated ARI decreased from 14.8 ± 1.3 to 8.1 ± 1.9 days; $p \leq 0.05$ (Fig. 1).

The microbiological oropharyngeal examination detected some pathogenic and opportunistic microorganisms in 92.0% of children in the period of relative clinical well-being: *St. aureus* – 68.0%, *Str. haemolyticus* – 48.0%, *Str. pyogenes* – 52.0% or associations of coccal flora or their combination with *E. coli* – 18.0%, *C. albicans* – 34.0% and *P. aeruginosa* – 10.0%. The persistence of the potentially pathogenic bacteria in the oropharynx should be considered as the indicator of the exhausted local defense mechanisms determining the risk of the chronic processes associated with the increased viral load.

The oropharyngeal microbiocenosis parameters after completion of the specified courses were significantly improved: number of children with the excessive and moderate growth of the opportunistic flora and associations has significantly decreased (moderate and poor growth rates prevailed [grade III and II]), it was explained by the normalization of immunological processes secondary to the decreased antigenic viral load; $p < 0.01$ (Table 2).

Determined saliva microcrystallization types specified in Table 3 showed that type III of SMC was detected in 35.4% of children with ARRI indirectly indicating the decreased mineralizing function, saliva pH and its impaired antioxidant properties and explained the decreased protective functions of the respiratory tract mucous membranes secondary to the increased proliferative activity of the potentially pathogenic flora. Type I SMC corresponding to the standard level was detected only in 6.3% subjects from that cohort of children prior to the follow-up and at the end of the year – in 39.6%; $p < 0.01$

Table 2. Oropharyngeal microflora growth grade in the examined children over the time (n = 50)

Microflora growth grade	Prior to FLAVO VIR® syrup administration, n (%)	Post FLAVO VIR® syrup administration, n (%)	Changes of parameter over time
I	–	–	–
II	4 (8,0)	20 (40,0)	+32
III	10 (20,0)	26 (52,0)	+32
IV	36 (72,0)	4 (8,0)	-64
Comparison over the time $\chi^2=43,378$; $p<0,01$			

Note: $p < 0.05$ – statistically significant improvement of the parameter for the 5% level of significance according to the sign test

Table 3. Types of saliva microcrystallization in children with the recurrent respiratory infections (n = 48)

Type of saliva microcrystallization	Prior to FLAVO VIR® syrup administration, n (%)	Post FLAVO VIR® syrup administration, n (%)	Changes of parameter over time, abs. (%)
I type	3 (6,3)	19 (39,6)	+16 (33,3)
II type	28 (58,3)	17 (35,4)	-11 (22,9)
III type	17 (35,4)	12 (25,0)	-5 (10,4)
Comparison over the time $\chi^2=15,9$; $p<0,01$			

Discussion

The results of the conducted studies allow making a conclusion that the proposed prevention method with FLAVO VIR® syrup may be successfully used in children with the acute recurrent respiratory infections accompanied by the antioxidant imbalance. Using this method in the patients with ARRI allows decreasing incidence of diseases, their severity and duration which contributes to improving the quality of life in this cohort of patients. The method is easy-to-use, it does not cause the side effects and has high level of compliance; it may be used for ARI combination therapy both in the acute period to reduce the recovery time and in the remission period for the purpose of prevention and stronger control over the disease course and improved rehabilitation.

The obtained data make it possible to positively evaluate the organization of the preventive courses during the school period allowing the effective prevention and treatment of the respiratory diseases, decreasing severity of the acute disorders, rate of complications and hospitalizations, medication burden and preventing the chronic conditions in this group of children.

Conclusions

1. It was determined that using the medicinal product FLAVO VIR® (syrup) by the courses within a year for the treatment and preventive purpose results in the improved general condition of children, reduced manifestations of the intoxication and asthenic syndromes, ARI incidence and severity, number of days missed from school, number and duration of hospitalizations ($p < 0.01$).

2. Type III of saliva microcrystallization was detected in 35.4% of children with ARRI indicating the decreased mineralizing function, saliva acidity (pH), its impaired antioxidant

properties and explaining the decreased protective functions of the respiratory tract mucous membranes secondary to the increased proliferative activity of the potentially pathogenic flora (*St. aureus*, *Str. haemolyticus*, *Str. pyogenes*, *E. coli*, *C. albicans*, *P. aeruginosa*). FLAVOVIR® syrup used in this group of children facilitates the enhanced mineralizing potential of saliva (in 39.6% of children – type I SMC) secondary to the decreased oral microbiological load ($p < 0.01$).

3. The antiviral and antioxidant effects together with the interferonogenic properties make the use of FLAVOVIR® syrup reasonable both in the treatment of the acute recurrent respiratory infections in children and in the preventive courses in the children with ARRI. FLAVOVIR® used for the preventive and therapeutic purpose is well tolerated by the children; it does not cause the complications, development or exacerbation of the allergic reactions explaining high level of compliance.

References:

1. Abaturon A. Ye., Vysochina I. L. Realization of antiviral and antioxidant effects of bioflavonoids in treatment of acute respiratory viral infections. *Zdorov'ye Rebenka*. 2016;5(73):42–8.
2. Beketova G. V., Khaitovich N. V., Grynevych O. I. Immunoflazidum in pediatrics: system analysis of effectiveness and safety. *Pediatrija. Vostochnaja Evropa*. 2014;2(07):141–52.
3. Gonchar F. L., Pokhodenko-Chudakova I. O. Microcrystallization of oral fluid as a general homeostasis parameter. In: *Proceeding of Republic Scientific Research Conference: Innovative approaches to practical decisions of topic problems in maxillofacial surgery and dentistry*. Minsk; 2010. p. 70–2.
4. Leus P. A. Diagnosticheskoe znachenie gomeostaza sljunny v klinike terapevticheskoy stomatologii : uchebno-metodicheskoe posobie [Diagnostic value of saliva homeostasis in clinical picture of therapeutic dentistry: textbook]. Minsk: Belorussian State Medical University; 2011. 67p.
5. Kobiyasova I. V., Savushkina N. A. Features of mineralizing saliva function in adolescents of pubertal age and methods for its correction. *Sovremennaya stomatologiya*. 2006;2:64–7.
6. Mukvich O. M., Kaminska T. M. Organization of preventive-rehabilitation measures at school. *Sovremennaya pediatriya*. 2016;3(75):15–18. doi: 10.15574/SP.2016.75.15.
7. Nedilko V. P., Kaminska T. M., et al. Ways for improvement of health in schoolchildren. *Sovremennaya pediatriya*. 2010;3(31):81–4.
8. Rybalko S. L. Otchet «Izuchenie mekhanizmov dejstvija biologicheskii aktivnih veshhestv lechebnoj substancii Proteflazid» [Report] Study of mechanisms of biologically active substances of remedial Proteflazidum]; 2010. 84p.
9. Yulish Ye. I., Ivanova L. A., Chernyshova O. E. Immune impairments in sickly children and chronic children. *Pediatrija, akusherstvo ta ginekologiya*. 2003;4:109.
10. Hosakote Y. M., Liu T., Castro S. M., et al. Respiratory syncytial virus induces oxidative stress by modulating antioxidant enzymes. *Am J Respir Cell Mol Biol*. 2009 Sep;41(3):348–57. doi: 10.1165/rmb.2008-0330OC.
11. Hosakote Y. M., Jantzi P. D., Esham D. L., et al. Viral-mediated inhibition of antioxidant enzymes contributes to the pathogenesis of severe respiratory syncytial virus bronchiolitis. *Am J Respir Crit Care Med*. 2011 Jun 1;183(11):1550–60. PMID: 21471094. PMCID: PMC3137144. doi: 10.1164/rccm.201010-1755OC.
12. Lyu S. Y., Rhim J. Y., Park W. B. Antiherpetic activities of flavonoids against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) *in vitro*. *Arch. Pharm Res*. 2005 Nov;28(11):1293–301. PMID: 16350858.

13. de Martino M., Ballotti S. The child with recurrent respiratory infections: normal or not? *Pediatr Allergy Immunol.* 2007;18 Suppl 18:13-8. doi: 10.1111/j.1399-3038.2007.00625.x. PMID: 17767600.
14. Stensson M., Wendt L. K., Koh G., et al. Oral health in pre-school children with asthma – followed from 3 to 6 years. *Int J Paediatr Dent.* 2010;20(3):165-72. PMID: 20409196. doi: 10.1111/j.1365-263X.2010.01037.x.
15. Ozcelik B., Kartal M., Orhan I. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. *Pharm Biol.* 2011;49(4):396-402. PMID: 21391841. doi: 10.3109/13880209.2010.519390.
16. Gescher K., Kühn J., Lorentzen E., et al. Proanthocyanidin-enriched extract from *Myrothamnus flabellifolia* Welw. exerts antiviral activity against herpes simplex virus type 1 by inhibition of viral adsorption and penetration. *Journal of Ethnopharmacology.* 2011 Mar 24;134(2):468-74. PMID: 21211557. doi: 10.1016/j.jep.2010.12.038.
17. Odenthal J., van Heumen B. W., Roelofs H. M., et al. The influence of curcumin, quercetin, and eicosapentaenoic acid on the expression of phase II detoxification enzymes in the intestinal cell lines HT-29, Caco-2, HuTu 80, and LT97. *Nutr. Cancer.* 2012;64(6):856-63. PMID: 22830632. doi: 10.1080/01635581.2012.700994.

Reference: O. M. Mukvich, T. M. Kaminska. *Preventive and therapeutic effectiveness of bioflavonoids in children with recurrent respiratory infections // Clinical Pediatrics. – 2017. – Volume 12, No. 2. – pp. 124–129.*

1.6. FLAVOVIR® IN PEDIATRICS: A SYSTEMATIC ANALYSIS OF THE EFFECTIVENESS AND SAFETY OF USING

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Abstract

Based on the results of 12 clinical trials involving more than 800 children, conducted a systematic analysis of the efficacy and safety of domestic drug FLAVOVIR® for the treatment of viral and viral-bacterial infections in children of different age groups on the background suppression of the immune system. Analyzed in this work, scientific publications support the clinical efficacy and good safety profile syrup FLAVOVIR® when assigning children with therapeutic and prophylactic purposes.

Key words: FLAVOVIR®, flavonoids, children, spreading of respiratory desists, flu, immune system, clinical research

Introduction

Acute respiratory viral infections (ARVI) including influenza are the most common infectious pathologies of childhood accounting for up to 90% of all infectious diseases. ARVI is caused by more than 300 different types of viruses. The high incidence of ARVI is explained by the significant variety of pathogens: influenza (A, B, C), parainfluenza, adenovirus, respiratory syncytial virus, rhinovirus, enterovirus and other viruses [1]. In addition to the direct etiological causes of ARVI, some factors facilitating the development of recurrent episodes of the respiratory infection in children should also be mentioned; they include such important aspects as unfavorable premorbid background, perinatal central nervous system damages, intrauterine infection, immaturity, prematurity, etc. They have negative effect on all systems of the developing body and especially on the full development of immunity [2].

It has been proven that during the whole period of childhood, the immune system matures and develops; therefore, the so-called “critical” periods are distinguished that are characterized by the specific features of its functioning. The neonatal period is considered to be “critical” (the child’s body is protected almost exclusively by the maternal antibodies obtained through the placenta and with breast milk); 4–6 months of life when a child has the sharp decrease of the maternal antibodies; the 2nd year of life (the child’s contacts with the outside world and infectious agents are significantly expanded); the 6th–7th years of life (the child’s blood has the low absolute and relative lymphocyte count, level of immunoglobulin A, blood levels of immunoglobulins M and G are approaching normal values and immunoglobulin E content reaches its maximum) (Fig. 1); the adolescent period when a growth spurt is combined with the relative decrease of the lymphoid organ weights [3].

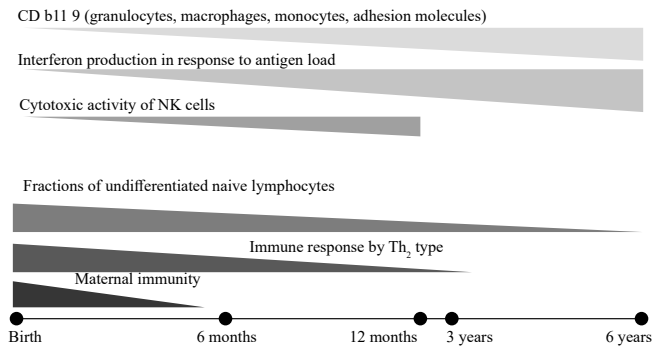


Figure 1. Development of anti-infective protection in children

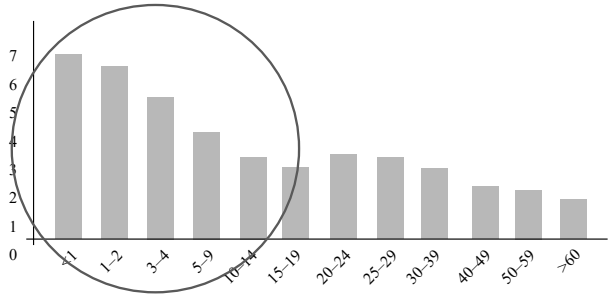


Figure 2. Mean incidence of respiratory infections within a year based on the patient age (Heikkinen et al., 2003)

Among the newborns, the group at high risk of infectious diseases includes the premature infants and children born with the low and extremely low body weight. They have marked immature immune system. The second “critical” period is characterized by the poor synthesis of only immunoglobulin M and insufficient local protection of the mucous membranes due to the later accumulation of secretory immunoglobulin A. In the children aged 1 to 6 years, a group with the “late start of immunological development” is distinguished; these children are characterized by the repeated or recurrent acute respiratory viral infections. According to the data from the cohort studies conducted by Finnish researchers (Figure 2), the highest incidence of the recurrent ARVI (up to 5–7 episodes per year) is characteristic exactly for the age of 1 to 6 years. From the physiological point of view, it is reasonable, as if a child has a large number of contacts with the infectious agents, frequent ARVI episodes are the “price” that the child “pays” for the full maturation of the immune system. Therefore, age of 6 months to 6 years is characterized by the increased susceptibility of a child to ARVI and is the characteristic feature of its ontogenesis. However, if the ARVI is prolonged and complicated, the immune system is incapable to protect the body from the infectious agents – when “the viruses impose a sentence and bacteria execute it” [4].

It should be noted that the clinical and laboratory markers of the immune deficiency in such children are the high incidence of ARVI recurrences (more that 6 times per year) with the prolonged or complicated disease course; weak but long-term fever response (prolonged subfebrile condition); low efficacy of the conventional treatment; presence of focal chronic ENT infection; persistent leucopenia,

neutrocytopenia, lymphocytopenia or unmotivated lymphocytosis; low ESR during the acute period of the disease, especially in the bacterial infections. The immunological markers of the impaired anti-infectious protection are: deficiency of IgA (sIgA) and factors of cell-mediated immunity; decreased activity of phagocytosis, NK and neutrophil chemotaxis; insufficient synthesis of interferons.

In the settings of the inflammatory process in ARVI, the following is also noted: increased production of the reactive oxygen species ensuring the bactericidal activity of neutrophils, development of oxidative stress further leading to depletion of the antioxidant system and cascade-like damage of the cell membranes not only of the affected organ, but also of the immune system [5]. Therefore, in addition to the conventional methods of treatment and rehabilitation of sick children, it is often necessary to prescribe the medicinal product that have an effect on the immunity and ensure the antioxidant effects.

The studies of the antiviral, antioxidant and immunomodulatory properties of flavonoids have been conducted globally for a long time. The flavonoids comprise a group of the natural biologically active compounds – benzopyrone derivatives based on the phenylpropane skeleton consisting of $C_6-C_3-C_6$ carbon units with an oxygen atom in the heterocyclic ring. Based on the degree of oxidation and hydroxylation of the propane skeleton and the location of the phenyl radical, the flavonoids are divided into several groups: flavones (chrysin, apigenin), isoflavones, flavonols, flavanones and flavanonols.

The results of the experimental studies and clinical trials confirmed the efficacy of the flavonoids in the prevention of the respiratory, immune, cancer, neurodegenerative, cardiovascular and other diseases.

Flavonoids are predominantly poorly soluble in water and can be accumulated in the lipid layer of biological membranes. The mechanism of action of most flavonoids is explained by the interaction with the various receptors and by the effect on their function. The flavonoids exert the antioxidant effects by altering the cellular metabolism.

The recent studies suggest that complexes of flavonoids with transition metal cations are involved in the formation of the molecular assemblies; they facilitate the membrane adhesion and fusion, protein-protein and protein-membrane binding and other processes responsible for the regulation of the cellular meta-environment [6].

The efficacy and safety of the domestic antiviral product from the group of bioflavonoids – FLAVOVIR® syrup (SMC “Ecopharm”, Kyiv) has been actively studied within the last 10 years. Each 100 ml of syrup contain 2 ml of Proteflazid liquid extract derived from the mixture (1:1) of *Herba Deschampsia caespitosa* L. and *Herba Calamagrostis epigeios* L. grasses; 1 ml of extract contains not less than 0.32 mg/ml of flavonoids equivalent to rutin. The flavonoids contained in FLAVOVIR® syrup belong to the groups of flavone and flavonol glycosides.

Table 1. Dosage regimen (ml) and dosage frequency of the medicinal product

Patient age (years)	Dosing (ml) and dosage frequency
from birth to one year	0.5 ml twice daily
1–2 years	1 ml twice daily
2–4 years	3 ml twice daily
4–6 years	4 ml twice daily
6–9 years	5 ml twice daily
9–12 years	6 ml twice daily
older than 12 years	9 ml twice daily

Herba Deschampsia caespitosa L. and Herba Calamagrostis epigeios L. are the wild plants harvested in the clean ecological areas of Volyn, Ivano-Frankivsk, Lviv, Zakarpattia regions (cleanliness of the ecological areas is determined by the tests for herbicides, pesticides and radiological contamination).

The herbal raw material is harvested according to the GMP rules with issuing the health certificates for each batch. The collection of raw materials is determined by the dynamics of replenishment of the active substances during the growing season.

The extraction is performed without chemical and biological factors and without the use of the intensive extraction methods, instead using the static trial maceration method.

As FLAVOVIR® has a high safety profile, it is allowed to be used in children from birth. The age-based dosage regimen of FLAVOVIR® syrup for children is presented in Table 1.

It has been proven that flavonoids contained in FLAVOVIR® (syrup) inhibit the replication of the respiratory viral DNA and RNA, including influenza viruses both *in vitro* and *in vivo*. The mechanism of the antiviral effect of the medicinal product involves the virus-specific enzyme inhibition – DNA polymerase, thymidine kinase and reverse transcriptase; anti-influenza effect – inhibition of neuraminidase activity, induction of the endogenous α - and γ -interferon synthesis, inhibition of RNA virus synthesis. Potentiating the action of the apoptosis-inducing substances, the medicinal product promotes faster elimination of virus-infected cells and prevention of the chronic diseases secondary to the latent viral infections.

In 2010, the study was conducted to investigate the efficacy of flavonoids towards the pandemic agent of type A influenza on the animal model of influenza pneumonia. Study results demonstrated that the medicinal product reduced the infectious titer of influenza virus (H1N1/California) in the lung tissues of infected animals by 250 times [7].

The results of the clinical studies proved that when the medicinal product FLAVOVIR® is used for a long time, it does not inhibit the activity of α - and γ -interferon production resulting in the normalization of the patient's immune status [8, 9, 28].

FLAVOVIR® protects the mucous membranes of the upper respiratory tract normalizing the local immunity parameters (lactoferrin, sIgA and lysozyme); enhances the antioxidant status of cells by inhibiting the free-radical processes and preventing the accumulation of the lipid per-oxidation products, decreases the intoxication, promotes the body recovery after the infection and adaptation to the adverse environmental factors [9].

We performed the analysis of the results of studies of the medicinal products containing active substances flavonoids that are the components of FLAVOVIR® syrup – 12 clinical studies (including 10 randomized) in children. In total, more than 800 children who received flavonoids for the therapeutic and preventive purpose took part in the studies (Table 2).

The preventive efficacy of FLAVOVIR® syrup in the influenza and ARVI was studied in 220 children (aged 1 to 6 years) attending the day care centers. The followed-up children were divided into two groups (110 subjects in each) [2]. 14-day preventive course with FLAVOVIR® syrup used in the period of seasonal epidemics allowed decreasing ARVI incidence in the study group by 2.2 times ($p < 0.05$). No complications were observed in the sick children from the study group. Days missed from the day care centers due to ARVI per one child in the study group were decreased by 35%, i.e. almost by 2 days.

Based on the results of the clinical study [10], preventive use of flavonoids in the toddlers and preschoolers from the kindergarten (residents of a large industrial center) allows decreasing ARVI incidence by 1/3.

Analysis of ARVI incidence in the young children group who received FLAVOVIR® as the preventive course compared with the control group demonstrated that during the first week 6 (22.2%) children fell ill with ARVI 6 (22.2%) in the study group and 14 (56.0%) children in the control group. Severe disease in the study group was observed half as often compared with the control group and disease duration was 3-fold shorter (on average 3.3 ± 0.7 days versus 9.7 ± 1.6 days) [11].

Another study showed that 67% of patients who received FLAVOVIR® syrup did not fall ill with influenza and ARVI during the epidemic period and children with the mild and abortive disorder without complications were prevailing among the sick ones [12].

In the children with the severe complicated ARVI who received FLAVOVIR® syrup in addition to the dosage regimen [1, 13], serum thymulin level was elevated by 53.6% ($p < 0.05$), while in the control group it was decreased by 32.3% ($p < 0.05$). In the children from the study group, CD⁺ lymphocytes (CD3⁺, CD4⁺, CD8⁺, CD16⁺) and immunoregulatory index CD4/CD8 were normalized. In particular, level of CD4⁺ T-helpers in the study group was increased from $35.1 \pm 1.2\%$ to $42.7 \pm 1.2\%$ ($p < 0.05$). Also, following the administration of FLAVOVIR® syrup, significant increase of the cell-phagocytic protection coefficient and decrease of the specific immune lymphocyte-monocyte potential (from 0.598 ± 0.05 to 0.701 ± 0.03 and from 0.832 ± 0.54 , respectively) ($p < 0.05$) were observed, while in the control group there were no positive changes.

In the patients from the study group, the period of the hospital stay was 1.5-fold shorter than in the control group (6.02 ± 0.5 days versus 12.6 ± 0.7 days, $p < 0.05$); the disease was milder and fever period was twice shorter (4.1 ± 0.6 days versus 8.7 ± 0.7 days, $p < 0.05$); intoxication symptoms were less marked and resolved twice as fast (4.6 ± 0.3 days versus 10.6 ± 0.7 days, $p < 0.05$) [1, 13].

When FLAVOVIR® syrup was added to the combination therapy of the young children with ARVI, the good clinical effect was observed in 90% of the sick children – decreased respiratory symptoms, the temperature persistently returned to normal values within 2–3 days after the medicinal product was prescribed. Mean treatment duration of children with ARVI was 6.5 days. At the end of the first week, the intoxication signs were observed only in 2 patients but their general condition was improved. Clinical manifestations of rhinitis and rhinopharyngitis decreased in all examined children; rare productive cough remained in 7% of children only [14].

The high antiviral efficacy of flavonoids was detected in treatment of 65 patients with the recurrent respiratory diseases. According to the results of 3-month therapy course, ARVI incidence within a year was lower by almost 1.5 times and complications rate was lower by 1.4 times. A year later, along with the normalization of immunity parameters, ARVI incidence was decreased by nearly 3 times; their complications were reduced by 35%; duration of disease episode was shorter by 1.5 times. The authors substantiated the need for the repeated course of the combined etiopathogenetic therapy 2 times per year with a 6-month interval [15].

Based on the results of flavonoids use, the signs of activated antiviral immunity were recorded; it was confirmed by the α -interferon level increased following the therapy course (from 10.25 ± 1.26 pg/ml to 39.89 ± 5.24 pg/ml, $p < 0.001$). The reduction of the inflammatory activity was confirmed by the decreased pro-inflammatory cytokines, in particular TNF (from 84.37 ± 28.61 pg/ml to 4.20 ± 2.90 pg/ml, $p < 0.001$) indirectly indicating the decreased antigenic load due to the inhibition of virus replication. Decreased concentration of IL-2 (from 187.34 ± 58.41 pg/ml to 28.17 ± 15.12 pg/ml, $p < 0.001$) and IL-6 (from 24.85 ± 4.65 pg/ml to 16.09 ± 8.45 pg/ml, $p < 0.001$) indicated the restoration of the cytokine

balance and decrease of the inflammation activity. The authors believe that the normalization of the anti-inflammatory IL-4 levels (from 54.18 ± 13.25 pg/ml to 14.35 ± 7.98 pg/ml, $p < 0.001$) also demonstrates the achievement of the relative cytokine balance and the subsidence of the inflammatory process, which from being mild (characteristic of the infection persistence) acquires the features of the acute process indicating the adequate immune response to the foreign agents [9].

Table 2. Efficacy and safety of FLAVOVIR® syrup used in children in the therapy of diseases accompanied by the immune suppression

Source	Subjects	Objective	Efficacy	Tolerability
Yulish Ye. I., Soroka Yu. A. et al., 2009 [7]	110 children aged 1 to 6 years	Prevention of ARVI	ARVI incidence was decreased by 2.2 times, there were no complications, days missed from the child care institutions were decreased by 35%	Good
Reznichenko Yu. G. et al., 2008 [8]	30 children of preschool age	Prevention of ARVI	ARVI incidence in preschoolers was decreased by 1/3	Good
Yulish Ye. I., Balychevtseva I. V. et al., 2009 [9]	55 young children	Treatment and prevention of ARVI	Incidence was decreased by 2.3 times, disease duration was decreased by 3 times, there were no severe cases	Good
Bazarenko V. I., Ovchynnykova N. M., 2009 [10]	218 children	Treatment and prevention of ARVI	67% of children did not fall ill in the epidemic period. Sick children had the abortive or mild severity without complications	Good
Tokarchuk N. I., Starynets L. S., 2012 [11, 12]	25 young children	Treatment of ARVI	Thymulin level was increased by 53.6%, phagocytic protection coefficient was increased. Period of the hospital stay was shorter by 1.5 times, fever and intoxication duration was 2-fold shorter	Good
Sichnenko P. I. et al., 2008 [13]	120 young children	Treatment of ARVI	Positive clinical effect was observed in 90% of sick children secondary to Iminoflazid administration	Good
Yulish Ye. I., Yaroshenko S. Ya., 2013 [14]	65 young children	ARVI prevention in children who fall ill often and for a long time in herpesvirus infection	ARVI incidence was decreased by 1.5 times within a year (further on – by 3 times), complications were decreased by 1.4 times, disease episode duration was decreased by 1.5 times	Good
Prokhorova M. P. et al., 2008 [16]	35 children aged 2 to 7 years	Treatment of ARVI with obstructive syndrome	Positive immunological changes over the time were observed, recovery occurred on Day 5	Good
Tokarchuk N. I. et al., 2011 [17, 18]	25 young children	Treatment of community-acquired pneumonia secondary to herpesvirus infection	Duration of the hospital stay was shorter by 6.6 ± 1.3 days	Good
Tsymbalista O. L., Haridzhuk L. I., 2011 [19]	40 young children	Treatment of children with complicated pneumonia secondary to iron deficiency anemia	The level of pro-inflammatory cytokines was decreased by 2–6 times	Good

Motsar V. V. et al., 2011 [20]	30 young children	Protein-energy deficiency	Body weight of children who received Iminoflazid was increased twice as much compared with the reference group	Good
Hodovanets O. I., Rozhko M. M., 2007 [21]	48 young children	Prevention and treat- ment of chronic catarrhal gingivitis in children living in the nitrate pollution conditions	Intensity of the clinical manifesta- tions was decreased by 2–3 times, probability of disease recurrence was decreased by 2 times	Good

Use of flavonoids (FLAVOVIR®) in 35 children aged 2 to 7 years with ARVI and broncho-obstructive syndrome showed the significant reduction of blood CD4+, CD8+, CD16+ levels secondary to the decreased CD22+ values and IgG, IgM levels [16].

The addition of FLAVOVIR® to the combination therapy in the young children with the community-acquired pneumonia resulted in decreased duration of hospital stay by 6.6 ± 1.3 days compared with the patients who received the basic therapy alone [17, 18]. The use of FLAVOVIR® syrup in combination with the standard treatment of the complicated pneumonia secondary to the iron-deficiency anemia (IDA) contributed to the decreased blood pro-inflammatory cytokines in the patients: TNF (by 2.2–2.6 times); IL-6 (by 1.6–2.0 times) [19].

After 2-week therapy with added FLAVOVIR® syrup at the therapeutic dose in the young children who received inpatient treatment due to the protein-energy deficiency caused by the intrauterine infection and inhibiting function of the thymus gland, lymphocytes and spleen, 80% of patients demonstrated the increased body weight by more than 400.0 g, while in the reference group the body weight was increased only by 43.3%. Blood IgA level normalized in 90% of children from the study group and only in 36.6% in the control group [20].

After the administration of the medicinal product FLAVOVIR® in the young children, the following effects were observed: increased activity of the antioxidant protection system in the oral mucosa cells and accelerated regression of clinical manifestations of the chronic catarrhal gingivitis (CCG) [21–24]. Levels of diene conjugates and malondialdehyde in the children from the study group with the mild to moderate CCG were lower by 12.5–11.1% and 19–24.6%, respectively compared with the reference group. The activity of the antioxidant enzyme catalase in the mild CCG was 30.8% higher and in moderate CCG it was 47.2% higher in the follow-up group compared with the control group. The post-treatment level of HS groups and glutathione was significantly higher in the children from the study group compared with the children from the reference group (by 62.2% and 54.8% in the mild CCG and 67.1% and 66.0% in the moderate severity), which was also reflected in the activity of glutathione-dependent enzymes: activity of glutathione reductase and glutathione-S-transferase in the follow-up group was higher by 40.2% and 67.7% in the mild severity and by 56.1% and 82.9% in the moderate severity compared with control group.

One month later, on Day of treatment 2–3, in the mild CCG, the significant clinical improvement and resolution of the inflammation signs were observed (mean duration of topical treatment was 4.30 ± 0.26 days versus 6.30 ± 0.23 days in control group); in the moderate disease, in the children from the study group, the hyperemia, edema and bleeding regressed starting from Day of treatment 3–4 and the duration of correction was 6.20 ± 0.21 days versus 9.30 ± 0.51 day in the conventional therapy. The physical examination of children following (14-day) therapy course showed the complete elimination of the gingival pathological process secondary to the flavonoids

use. The inflammation signs in the reference group were preserved in 75.0% of children with the mild disease and in 100.0% of children with the moderate disease.

One month later, in the mild CCG, the disease recurrence without gingival bleeding was observed in 41.7% and in moderate severity – in 50.0% the children from the study group. In the reference group, CCG recurrence was observed in 100.0% of subjects. Therapy course with flavonoid products decreased the intensity of the clinical manifestations in the recurrent disease after 6-month follow-up and 2-fold and 3-fold in children with the mild and moderate CCG.

The flavonoid products are used as a several-week course for the treatment of viral diarrheas. If the bacterial complications are developed, the medicinal product is used for up to 4 weeks to normalize the immune system parameters. For the prevention of the viral diarrheas, for example, in the summer or during the increased viral diarrhea incidence, the flavonoid products are used for 2 to 4 weeks at the dose corresponding to half the therapeutic one [25].

The combination therapy of children with the recurrent respiratory pathology using FLAVOVIR® (syrup), reduced the ARVI incidence and duration in children by almost 7 times by harmonizing the adaptive reactions and improving the functions of the epithelial system [26].

Many clinical studies have been devoted to the medicinal product FLAVOVIR® (syrup); their results are partially presented in the literature review prepared by N. P. Hliadelova [27]. The scientific publications analyzed in this article indicate the good clinical efficacy of FLAVOVIR® syrup and other medicinal products containing equivalent flavonoids (PROTEFLAZID®) and high safety profile when prescribed to children for the purpose of treatment and prevention in ARVI.

Conclusions

The medicinal product FLAVOVIR® (syrup) used at the standard therapeutic doses has the direct antiviral action and exerts the immunomodulatory effect, reduces the level of pro-inflammatory cytokines, stimulates T-helper activity increasing the level of nonspecific protection and decreases the development of oxidative stress. High efficacy of the retreatment courses using FLAVOVIR® syrup was demonstrated in the recurrent viral infections.

When FLAVOVIR® syrup is used for prevention purpose, it decreases the influenza and ARVI incidence in preschoolers by 1/3, minimizes the days missed at the child care institutions by 35%, decreases the ARVI incidence in the young children by 2.2–2.3 times and prevents the severe diseases. When FLAVOVIR® syrup is added to the combination therapy of the patients with the severe ARVI and pneumonia, the hospitalization period is reduced by 6.6 days and intoxication and fever period – two-fold. When FLAVOVIR® syrup is added to the combination therapy of the children with the protein-energy deficiency and chronic catarrhal gingivitis, significantly accelerated improvement was observed.

It should be noted that when FLAVOVIR® syrup is used daily for a long time, it demonstrates the high safety profile in the children [8, 9, 28].

The therapeutic effects of the medicinal product FLAVOVIR® (syrup) give ground to recommend its broad use in the children with influenza and ARVI. FLAVOVIR® is the product that completely meets the requirements for ARVI treatment and prevention in children [1, 13].

Based on the results of all provided studies, the good tolerability and appropriate compliance was observed when using FLAVOVIR® syrup.

Thus, the systemic analysis of the conducted clinical studies (involved more than 800 sick children) confirmed the efficacy and high safety profile of the herbal medicinal product

FLAVOVIR® (syrup) used for prevention and treatment of influenza and ARVI and also their complications in the children from different age groups and gives the reason to recommend this medicinal product for its broad use.

References:

1. Токарчук Н. І. Досвід використання імунофлазиду в комплексі лікувально-профілактичних заходів під час сезонного підвищення захворюваності на грип та ГРВІ / Н. І. Токарчук, Л. С. Старинець // Перинатологія та педіатрія – 2012. – Т. 49, №1. – С. 1–5.
2. Юліш Є. І. Ефективність імунофлазиду у профілактиці гострих вірусних інфекцій у дітей дошкільного віку / Є. І. Юліш // Сучасна педіатрія – 2009 – Т. 26, №4. – С. 100–101.
3. Фрейдлін І. С. Особливості імунітету в дітей різного віку. Доступний на <http://www.licopid.ru/osobennosti-immuniteta-u-detey-raznogo-vozrasta>.
4. Романцов М. Г. Діти, що часто хворіють: сучасна фармакотерапія: кер-во для лікарів / М. Г. Романцов. – М., 2006. – 189 с.
5. Дідковський Н. А. Принципи імунокоригуючої терапії при інфекційно-запальних захворюваннях, 2009. http://www.rlsnet.ru/articles_402.htm.
6. Tarahovsky Y. Flavonoid-membrane interactions: involvement of flavonoid-metal complexes in raft signaling / Y. Tarahovsky [et al.] // Biochim Biophys Acta. – 2014. – Vol. 1838 (5). – P. 1235–1246.
7. Звіт про НДР «Експериментальне вивчення протівірусної ефективності препарату Протефлазид щодо збудника грипу А (H1N1)v на моделі грипозної пневмонії у тварин» / Міністерство охорони здоров'я та соціального розвитку Російської Федерації, Науково-дослідний Інститут грипу (ФДБУ НДІ). – СПб., 2010.
8. Панасюк О. Л. Етіопатогенетична терапія герпесвірусної інфекції із застосуванням протефлазиду та ультрафіолетового опромінювання крові: автореф. дис. ... канд. мед. наук. 14.01.13 – інфекційні хвороби/О. Л. Панасюк; Інститут епідеміології та інфекційних хвороб ім. Л. В. Громашевського. – К. – 180 с. Доступно на <http://www.lib.ua-ru.net/diss/cont/248481.html>.
9. Юліш Є. І. Діти, що часто хворіють, і тактика педіатра / Є. І. Юліш, С. Я. Ярошенко // Здоров'я дитини – 2013. – Т. 49, №6 – С. 101–108.
10. Резніченко Ю. Г. Пошук шляхів профілактики гострих респіраторних захворювань у дітей- мешканців великого промислового міста / Ю. Г. Резніченко, Р. Л. Шевченко, В. І. Безсікало // Сучасна педіатрія. – 2008. – Т. 19, №2. – С. 49–50.
11. Юліш Є. І. Нові підходи до профілактики та терапії гострих респіраторних інфекцій у дітей раннього віку, які проживають у будинках дитини / Є. І. Юліш [та ін.] // Сучасна педіатрія – 2009. – Т. 25, №3. – С. 15–18.
12. Назаренко В. І., Овчиннікова Н. М. Досвід використання препарату «Імунофлазид» у лікуванні та профілактиці грипу та гострих респіраторних інфекцій / В. І. Назаренко, Н. М. Овчиннікова // Сучасна педіатрія – 2009. – Т. 23. – №1. – С. 1–2.
13. Токарчук Н. І. Використання Імунофлазиду для профілактики та лікування грипу та ГРВІ у дітей під час сезонного підвищення захворюваності / Н. І. Токарчук, Л. С. Старинець // Сучасна педіатрія – 2012. – Т. 41. – №1. – С. 123–127.
14. Січеннюк П. І. Застосування Імунофлазиду у лікуванні ГРВІ у дітей раннього віку / П. І. Січеннюк [та ін.] // Актуальні питання клінічної медицини. – 2009 – С. 88.
15. Юліш Є. І., Ярошенко С. Я. Персистуючі герпесвірусні інфекції та їх роль у захворюваності дітей із групи, де часто і довго хворіють / О. І. Юліш, С. Я. Ярошенко // Здоров'я дитини – 2013. – Т. 50 № 7 – С. 145–150.

16. Прохорова М. П. Ефективність препарату Флавозід® у дітей з ГРВІ, ускладненим бронхообструктивним синдромом / М. П. Прохорова // Актуальні питання педіатрії. – 2008. – №4.
17. Токарчук Н. І. Ефективність застосування імунофлазиду при лікуванні негоспітальної пневмонії у дітей раннього віку / Н. І. Токарчук [та ін] // Перинатологія та педіатрія. – 2011. – Т. 45, №1 – С. 63–65.
18. Залізюк А. А. Особливості сучасного перебігу негоспітальних пневмоній та їх лікування у дітей раннього віку: автореф. дис. на присвоєння наук. ступеня канд. мед. наук: спец. 14.01.10 «Педіатрія», 2011. – 23 с.
19. Цимбаліста О. Л. Динаміка маркерів запалення у дітей раннього віку, хворих на ускладнену пневмонію на тлі залізодефіцитної анемії / О. Л. Цимбаліста, Л. І. Гаріджук // Перинатологія та педіатрія – 2013. – Т. 56, №4. – С. 33–36.
20. Моцар В. В. Досвід застосування препарату «Імунофлазід» при білково-енергетичній недостатності у дітей раннього віку // Сучасна педіатрія. – 2011. – Т. 40, №6 – С. 63–64.
21. Годованець О. І. Ефективність застосування препаратів Протефлазід® та Імунофлазід® у комплексі лікування хронічного катарального гінгівіту у дітей / О. І. Годованець, М. М. Ріжко // Сучасна педіатрія – 2007. – Т. 16, №3. – С. 94–98.
22. Годованець О. І. Антиоксидантна терапія у комплексі лікування хронічного катарального гінгівіту у дітей, які проживають на нітратно-забруднених територіях / О. І. Годованець // Вісник Української медичної стоматологічної академії. – 2007. – Т. 7, №4. – С. 20–23.
23. Годованець О. І. Віддалені результати застосування препаратів антиоксидантної дії у комплексі лікування хронічного катарального гінгівіту у дітей // Світ медицини та біології. – 2012. – №3. – С. 80–83.
24. Годованець О. І. Особливості клінічного перебігу та лікування хронічного катарального гінгівіту у дітей, що проживають на територіях, забруднених нітратами: автореф. дис. ... ступеня канд. мед. наук: спец. 14.01.22. / О. І. Годованець // Стоматологія. – 2008. – С. 22.
25. Крамарьов С. О. Вірусні діареї у дітей: особливості клінічної картини, діагностика, сучасні підходи до терапії / С. О. Крамарьов [та ін] // Дитячий лікар. – 2014. – Т. 32–33, №3–4. – С. 25–32.
26. Папінко Р. М. Прогнозування перебігу та профілактика повторних рецидивних респіраторних захворювань у дітей: автореф. дис. на присвоєння наук. ступеня канд. мед. наук: спец. 14.01.10 / Р. М. Папінко // Педіатрія. – 2009. – 22 с.
27. Гляделова Н. П. Досвід застосування протівірусних препаратів рослинного походження у терапії та профілактиці грипу та інших гострих респіраторних вірусних інфекцій у дітей (огляд літератури) / Н. П. Гляделова // Сучасна педіатрія. – 2012 – Т. 45, №45. – С. 1–5.
28. Рибалко С. Л. Сучасний стан проблеми грипу А // Здоров'я нації. – 2010. – Т. 15, №3. – С. 169–178.

Reference: G. V. Bekeťova, M. V. Khaytovych, O. Y. Hrynevych. FLAVOVIR® in pediatrics: a systemic analysis of the effectiveness and safety of using // *Pediatrics. Eastern Europe*. – 2014. – No.3 (07). – pp. 141–152.

1.7. ANALYSIS OF THE CLINICAL TRIAL RESULTS OF THE FLAVONOID EFFECTS ON SOME CELLULAR IMMUNITY INDICATORS IN CHILDREN AND ADULTS

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Abstract

In the scope of this paper, an analysis of the clinical trial results assessing the clinical and immunological efficacy of the effects of flavonoids on some indicators of immunity in children and adults is given. The aim of the study was to evaluate the effects of flavonoids on the cellular component of the immune system in children and adults with viral and viral-bacterial diseases by using meta-analysis and statistical analysis of the clinical findings.

Materials and methods. The analysis comprises 5 scientific sources with the results of controlled clinical trials involving 412 children and 15 sources involving 1493 adult patients during the period from 2003 to 2017. In analysis Student's t-test was applied. Results. The data obtained indicate the efficacy of flavonoids in the treatment of viral and viral-bacterial diseases in children and adults. The administration of flavonoids (PROTEFLAZID®, FLAVOVIR®, Flavozid®) in the treatment of children with acute respiratory diseases and adults with HHV infection contributes to the normalization of cellular immunity indicators, the HHV infections symptom resolution, and the relapse rate reduction of genital herpes caused by HHV infection.

Conclusions. Therapy with such medical products as PROTEFLAZID®, FLAVOVIR®, Flavozid® helps to improve the clinical status and normalize the cellular immunity indicators in patients who were administered a treatment course.

Key words: PROTEFLAZID®, FLAVOVIR®, Flavozid®, flavonoids, meta-analysis, statistical analysis, herpes virus infection, immunity, clinical trials.

Introduction

One of the topical issues of contemporary world medicine is the high morbidity and mortality from viral infections occurring in the form of latent, acute, chronic, and slow persistent forms [1]. Viral and viral-bacterial diseases, first of all, acute respiratory viral infections (ARVI), including influenza, are characterized by multiple-organ tropism, causing damage to the respiratory system, gastrointestinal tract, cardiovascular, nervous, immune, and other systems. However, children, compared with adults, have a more severe disease course with the frequent serious complications and chronic pathology development [2].

Currently, it has been proved that all components of immunity are involved in protecting the organism from viruses, but its antiviral component has specific characteristics and includes a set of mechanisms directed at neutralizing and eliminating viruses, their antigens and infected cells. The immune defense of a macroorganism in viral diseases has peculiarities associated with two forms of the existence of the virus: extracellular and intracellular [3]. During the spread of viruses in cases when the virus integrates into the cell genome, cellular immune responses with the involvement of cytotoxic T-lymphocytes-killers [4] come to the fore that must be taken into account when creating antiviral agents.

At present, the issues of antiviral therapy are of great interest to doctors faced with the most common viral diseases (influenza, hepatitis, herpes and HIV infection). Among modern efficient agents with a high safety profile for the treatment of viral infections, there is a special group containing flavonoids – a group of natural biologically active compounds (benzopyrone derivatives), which are based on the phenylpropane skeleton, composed of the C₆-C₃-C₆ carbon units with an oxygen atom in the heterocyclic ring. Depending on the degree of oxidation, the propane skeleton hydroxylation and the phenyl radical location, flavonoids are divided into several groups: flavones (chrysin, apigenin), isoflavones, flavonols, flavonones, and flavononols [5].

Flavonoids have antiviral, antioxidant and immunotropic actions, confirmed by numerous experimental studies and clinical trials conducted over extended periods. Their efficacy in the prevention and treatment of respiratory, immune, oncological, neurodegenerative, cardiovascular and a number of other diseases has been proved [6]. PROTEFLAZID®, IMMUNOFLAZID® (FLAVOVIR®*) and FLAVOZID® represent a line of drugs containing a liquid extract of PROTEFLAZID® derived from the wild grass family *Herba Deschampsia caespitosa* L. and *Herba Calamagrostis epigeios* L., the active ingredient of which are flavonoids. The specific properties of these medications are determined by the fact that complex compounds of flavone O- or C-glycosides of tricine, apigenin, and luteolin act in the organism pharmacologically [6].

The agents have antiviral properties (inhibit virus-specific enzymes and neuraminidase of influenza viruses), immunomodulatory, antioxidant, and apoptosis-modulating actions [7].

The flavonoids contained in the agents have a direct antiviral effect on both DNA and RNA viruses, inhibit their replication, both *in vitro* and *in vivo*. When conducting preclinical and clinical studies, their antiviral effect against the viruses of herpes, hepatitis, papillomaviruses, HIV, influenza and acute respiratory infections has been revealed and proved. The mechanism of direct antiviral action of flavonoids involves the virus-specific enzyme inhibition – DNA and RNA polymerases, thymidine kinase, reverse transcriptase and neuraminidase of influenza viruses [10,11]. It has been established that the drugs are inducers of the endogenous α - and γ -interferon synthesis to a physiological level, increasing the host's non-specific resistance to viral and bacterial infections [8].

A distinctive feature of the drugs is that they do not cause the refractoriness of the immune cells (IC) with their long-term administration. Refractoriness (hyporeactivity) is understood as the inability of cells to respond with the interferon production to the repeated (multiple) introduction of the inducer. The lack of IC refractoriness to the introduction of PROTEFLAZID® and any drugs based on it is an important argument in choosing the drug,

* Editor's note: Flavozid® (syrup) has been out of production since 2019.

since in conditions of an immunological imbalance due to the pathogen effect, as well as taking antibiotics, it is essential to normalize the indicators of nonspecific humoral immunity as a triggering link in an immunological reaction [9,10]. Only under conditions of adequate functioning of the immune system, the efficacy of antiviral and antibacterial therapy and the pathogen elimination increase [10].

Research objective

To undertake the meta-analysis and statistical analysis of the clinical trial results assessing the effect of flavonoids (PROTEFLAZID®, FLAVOVIR®, FLAVOZID®) on the status of the cellular component of immune system in children and adults with viral and viral-bacterial diseases.

Materials and methods

Data sources and searching

The information on clinical trials studied the effect of flavonoids on the dynamics of the cellular immune component indicators in children and adults with viral and viral-bacterial diseases was searched on the Internet using information retrieval systems, as well as a literary search for publications of conference materials in the scientific and medical journals was made. The scientific articles reflecting the efficacy of flavonoids in the treatment of viral and viral-bacterial diseases were published during the period from 2003 to 2017.

The list of publications selected for the analysis, reflecting the clinical and immunological efficacy of the drug line containing the flavonoids of PROTEFLAZID® in children and adults with viral and viral-bacterial diseases (tab. 1 and 2).

Software. RevMan and SPSS. To ensure the reliability and accuracy of the results, calculations were made by two researchers.

Table 1. List of publications reflecting the efficacy of flavonoids (PROTEFLAZID®, IMMUNOFLAZID®, FLAVOZID®) in the treatment of children with viral and viral-bacterial diseases

Author	Total number of patients	Scope of drug application	Clinical results
Volosovets A.P. [12]	35	Acute respiratory viral infection with the obstructive syndrome development	Significant improvement of the indicators of the cellular and humoral component of immunity
Kriuchko T.O. [13]	65	Recurrent bronchitis in young children	Improvement of indicators of the cellular and humoral immunity, and phagocytic cell activity
Kuznetsova L.V. [14]	206	Influenza and acute respiratory viral infections in children	Improving the clinical condition of patients against the background of normalization of the cellular immunity indicators
Tokarchuk N.I. [15]	50	Community-acquired pneumonia in children	Normalization of indicators of the T-cellular component of immune system
Kharchenko Yu.L. [16]	56	Infectious mononucleosis in children	Reducing the febrile period and the intoxication severity

Table 2. Publications reflecting the efficacy of PROTEFLAZID® in the treatment of adult patients with viral and viral-bacterial infections

Author	Total number of patients	Scope of drug application	Clinical results
Baev A.I. [24]	140	Mixed STD	Improvement of the immunological and biochemical indicators
Bashmakov M.N. [25]	133	Immunorehabilitation of patients with purulo-necrotic complications on the background of diabetes mellitus	Normalization of immunological indicators
Gopchuk O.M. [26]	70	Menstrual disorders in women with herpes virus infection	A significant increase in the CD4 + lymphocytes and NK cells to normal values, an increase in IFH- γ and IFN- α
Grinkevich T.M. [27]	100	Genital herpes	Corrects imbalance between humoral and cellular components of immunity
Zapolskyi M.E. [28]	88	Multiform exudative erythema associated with herpes virus	Immunocorrecting, antioxidant, and detoxicative effect
Zelonyi I.I. [29]	88	Patients with erysipelas and purulo-necrotic complications	Helps stabilize immunological indicators
Klimenko P.M. [30]	40	Treatment of urogenital herpes	Improves the treatment efficacy, promotes the remission phase and reduces the disease relapse rate
Koveshnikov O.V. [31]	58	Rehabilitation of patients after laparoscopic cholecystectomy	Positive dynamics of immunological indicators
Krasnova S.P. [32]	186	Chronic pathology of the digestive organs	Subsidence of T-lymphopaenia, normalization of the T-lymphocyte subpopulations, normalization of the monocyte phagocytic activity
Leshchinskyi P.T. [33]	82	Chronic urogenital chlamydiosis	Normalization of the clinical and immunological indicators
Simrok V.V. [34]	65	Rehabilitation of women who have suffered an exacerbation of chronic salpingo-oophoritis	Reduction or subsidence of the autoimmune and immune complex-mediated reaction severity
Sundukov A.V. [35]	82	Herpes virus infection	Normalization of the main cellular immunity indicators
Udovika N.O. [36]	60	Chronic salpingo-oophoritis with a syndrome of increased fatigability	Reduction or subsidence of the severity of T-lymphopaenia, imbalance of the main regulatory T-cell subpopulations, decrease in the level of autoimmune reactions

Sotskaia Ya.A. [37]	124	Treatment of patients with chronic bronchitis against the background of recurrent angina	Accelerating the achievement of sustained and long-term disease remission in the examined patients, a significant decrease in the relapse rate of bronchitis with relapsed tonsillitis, as well as the normalization of immunological indicators
Khrushch V.I. [38]	177	Infected eczema and atopic dermatitis	Normalization of the cellular and humoral immunity and nonspecific defense factors

Inclusion and exclusion criteria

The trials intended for inclusion in the meta-analysis conform to the following criteria:

1. Controlled trials, providing for the presence of a control or a control group when studying the clinical and immunological efficacy of flavonoids (drugs: PROTEFLAZID®, IMMUNOFLAZID®, FLAVOZID®) in children and adults with viral and viral-bacterial diseases.

2. Mandatory confirmation of the clinical diagnosis of identified diseases in children and adults.

3. Published data evaluating the flavonoid efficacy (drugs: PROTEFLAZID®, IMMUNOFLAZID®, FLAVOZID®) are complete.

The trials to be excluded from the meta-analysis conform to the following criteria:

1. Non-controlled clinical trials assessing the clinical and immunological efficacy of flavonoids (drugs: PROTEFLAZID®, IMMUNOFLAZID®, FLAVOZID®) in children and adults with viral and viralbacterial diseases.

2. Doubling of previous publications. If more than one study by the same author of the same case series was published, the latest publication was included in the study.

3. Studies based on incomplete data.

4. No control group.

5. Meta-analyses, letters, reviews or editorials.

In accordance with the inclusion and exclusion criteria, 5 trials assessing the clinical and immunological efficacy of flavonoids in children were included in the analysis, 7 trials were excluded [17-23]; in adults – 15 trials were included in the analysis, and 12 were excluded [39-50]. In the controlled clinical trials included in the analysis, 412 children and 1493 adult patients were involved during the period from 2003 to 2017. Patients underwent a thorough examination prior to and on completing the therapy, including the routine clinical, laboratory and instrumental tests. To assess the immune status, the patients were provided a comprehensive immunological examination comprised the study of mature T-lymphocytes and their subpopulations, as well as B-lymphocytes.

A meta-analysis of clinical trials reflecting the results of the clinical and immunological efficacy of flavonoids (drugs: IMMUNOFLAZID®, FLAVOZID®) in children was conducted according to the statistically significant indicators which are as follows:

I. Dynamics of immunological indicators against the administration of flavonoids (IMMUNOFLAZID® and FLAVOZID®) in the treatment of acute respiratory diseases (ARD) in children:

- relative count of T-lymphocytes (CD3+) in the blood of children with ARD, %;
- relative count of T-helper-inducers (CD4+) in the blood of children with ARD, %;

II. Clinical benefit rate of the IMMUNOFLAZID® and FLAVOZID® administration in children with ARD.

The meta-analysis of clinical trials reflecting the results of the clinical and immunological efficacy of PROTEFLAZID® in adult patients was carried out according to the following statistically significant indicators:

I. Dynamics of immunological indicators against the PROTEFLAZID® administration in the treatment of viral and viral-bacterial diseases in adult patients:

- relative count of T-lymphocytes (CD3+) in the blood of adult patients, %;
- relative count of T-helper-inducers (CD4+) in the blood of adult patients, %;
- the value of the immunoregulatory index (CD4/CD8) in adults.

II. The relapse rate of genital herpes caused by human herpes virus infection (HHV infections).

III. Rate of resolution of HHV infections symptoms against the therapy with PROTEFLAZID®.

The clinical results on these indicators are presented in tables 3-11.

Table 3. Relative count of T-lymphocytes (CD3+) in children with ARD against the drugs IMMUNOFLAZID® and FLAVOZID® administration

Author	Treatment group		Control group	
	Relative count of T-lymphocytes (CD3+) in children with ARD who were administered IMMUNOFLAZID® and Flavozid®, %	Total number of patients	Relative count of T-lymphocytes (CD3+) in children with ARD who were not administered IMMUNOFLAZID® and Flavozid®, %	Total number of patients
Volosovets A.P., 2008	66,05±0,48	24	51,2±1,07	30
Kuznetsova L.V., 2017	69,4±2,2	51	49,2±2,1	46
Kriuchko T.O., 2009	47,4±2,3	21	37,0±2	20
Tokarchuk N.I., 2012	56,9±1,0	25	50,3±0,8	25
Total	-	121	-	121

On the indicator “Relative count of T-lymphocyte (CD3+) in children with ARD during the IMMUNOFLAZID® and FLAVOZID® administration at the stage of the treatment completion,” 242 children with ARD were examined, 121 of whom were enrolled to the treatment group and 121 – to the control group.

Table 4. Relative count of T-helper-inducers (CD4+) in children with ARD against the IMMUNOFLAZID® and FLAVOZID® administration

Author	Treatment group		Control group	
	Relative CD3+ count in children with ARD who were administered IMMUNOFLAZID® and FLAVOZID®, %	Total number of patients	Relative CD4+ count in children with ARD who were not administered IMMUNOFLAZID® and FLAVOZID®, %	Total number of patients
Volosovets A.P., 2008	38,5±0,33	24	29,3±1,30	30
Kuznetsova L.V., 2017	45,2±1,5	51	38,1±1,6	46
Kriuchko T.O., 2009	29,7±1,8	21	21,6±1,15	20
Tokarchuk N.I., 2012	42,7±1,2	25	38,6±1,1	25
Total	-	121	-	121

On the indicator “Relative count of T-helper-inducers (CD4+) in children with ARD against the IMMUNOFLAZID® and FLAVOZID® administration at the stage of the treatment completion”, 242 children with acute respiratory diseases were examined, 121 of whom were enrolled to the treatment group and 121 – to the control group.

Table 5. Clinical benefit rate of the IMMUNOFLAZID® and FLAVOZID® administration in children with acute respiratory diseases

Author	Treatment group		Control group	
	Number of children with ARD who were administered IMMUNOFLAZID® and FLAVOZID®	Total number of patients	Number of children with ARD who were not administered IMMUNOFLAZID® and FLAVOZID®	Total number of patients
Tokarchuk N.I., 2011	9	25	6	25
Kharchenko Yu.P., 2007	12	30	5	30
Total	21	55	11	55

On the indicator “Clinical benefit rate of the IMMUNOFLAZID® administration in children with acute respiratory diseases”, 110 children were examined, of whom 55 constituted the treatment group, and 55 – the control group.

Table 6. Relative T-lymphocytes (CD3+) count in patients with viral-bacterial diseases during the PROTEFLAZID® administration

Author	Treatment group		Control group	
	Relative CD3+ count in patients with viral-bacterial diseases who were administered PROTEFLAZID®, %	Total number of patients	Relative CD3+ count in patients with viral-bacterial diseases who were not administered PROTEFLAZID®, %	Total number of patients
Baev A.I., 2011	59,4±0,5	80	63,0±1,0	60
Bashmakov M.N., 2003	67,2±1,8	63	56,6±1,2	70
Krasnova S.P., 2003	67,5±2,1	96	56,3±1,6	90
Koveshnikov O.V., 2003	64,9±1,3	30	52,4±0,9	28
Khrushch V.I., 2016	65,22±2,09	33	64,57±2,18	32
Leshchinskyi P.T., 2003	68,6±1,8	42	56,4±1,6	40
Udovika N.O., 2003	72,3±1,3	32	65,2±2,1	28
Simrok V.V., 2004	72,2±1,4	37	65,2±2,1	28
Sotskaia Ya.A., 2003	68,6±1,9	65	59,9±1,8	59
Grinkevich T.M., 2005	62,88±0,69	20	60,11±0,63	20
Zelonyi I.I., 2003	68,5±2,1	46	54,9±1,8	42
Total	-	544	-	497

On the indicator “Relative count of T-lymphocyte (CD3+) in patients with viral-bacterial diseases against the PROTEFLAZID® administration at the stage of treatment completion”, 1,041 patients were examined, of whom 544 patients were enrolled to the treatment group, and 497 – to the control group.

Table 7. Relative T-helpers (CD4+) count in patients with viral-bacterial diseases against the PROTEFLAZID® administration

Author	Treatment group		Control group	
	Relative CD4+ count in patients with viral-bacterial diseases who were administered PROTEFLAZID®, %	Total number of patients	Relative CD4+ count in patients with viral-bacterial diseases who were not administered PROTEFLAZID®, %	Total number of patients
Bashmakov M.N., 2003	43,9±1,0	63	36,9±0,8	70
Baev A.I., 2011	45,8±0,5	80	42,8±1,0	60

Koveshnikov O.V., 2003	39,1±1,5	30	30,1±0,2	28
Krasnova S.P., 2003	44,3±1,5	96	35,7±1,3	90
Leshchinskyi P.T., 2003	45,1±1,1	42	34,6±1,1	40
Simrok V.V., 2004	42,6±1,6	37	40,0±1,4	28
Sotskaia Ya.A., 2003	45,2±1,3	65	34,4±1,2	59
Udovika N.O., 2003	42,8±1,4	32	40,1±2,3	28
Khrushch V.I., 2016	41,89±0,80	33	47,32±2,69	32
Zelonyi I.I., 2003	43,5±1,6	46	35,5±1,4	42
Total	-	524	-	477

On the indicator “Relative count of T-helpers (CD4+) in patients with viral-bacterial diseases against the PROTEFLAZID® administration at the stage of treatment completion”, 1,001 patients were examined, of whom 524 patients were included to the treatment group, and 477 – to the control one.

Table 8. The value of the immunoregulatory index (CD4/CD8) in patients with viral-bacterial diseases during the PROTEFLAZID® administration

Author	Treatment group		Control group	
	The value of CD4/CD8 in patients with viral-bacterial diseases who were administered PROTEFLAZID®, c.u.	Total number of patients	The value of CD4/CD8 in patients with viral-bacterial diseases who were not administered PROTEFLAZID®, c.u.	Total number of patients
Bashmakov M.N., 2003	2,08±0,03	63	1,77±0,02	70
Koveshnikov O.V., 2003	1,82±0,04	30	1,12±0,03	28
Krasnova S.P., 2003	2,02±0,03	96	1,7±0,03	90
Leshchinskyi P.T., 2003	2,0±0,04	42	1,5±0,03	40
Simrok V.V., 2004	1,93±0,25	37	1,81±0,5	28
Sotskaia Ya.A., 2003	2,0±0,05	65	1,5±0,03	59
Udovika N.O., 2003	1,98±0,23	32	1,81±0,5	28
Baev A.I., 2011	3,3±0,25	80	2,45±0,25	60
Khrushch V.I., 2016	2,13±0,38	33	2,70±1,32	32
Zelonyi I.I., 2003	1,9±0,03	46	1,63±0,04	42
Total	-	524	-	477

On the indicator “The value of the immunoregulatory index (CD4/CD8) in patients with viral-bacterial diseases against the PROTEFLAZID® administration at the stage of treatment completion”, 1,001 patients were examined, of whom 524 patients were included to the treatment group, and 477 – to the control one.

Table 9. Relapse rate of genital herpes caused by HHV infections

Author	Treatment group		Control group	
	Number of patients who were administered PROTEFLAZID® with the relapsed genital herpes	Total number of patients	Number of patients who were not administered PROTEFLAZID® with relapsed genital herpes	Total number of patients
Zapolskyi M.E., 2012	4	32	9	26
Sundukov A.V., 2008	10	22	24	30
Klimenko P.M., 2012	2	30	3	10
Grinkevich T.M., 2005	39	49	45	51
Total	55	133	81	117

On the indicator “Relapse rate of genital herpes caused by HHV infections”, 250 patients with relapsed genital herpes caused by HHV infections were examined, of whom 133 were enrolled to the treatment group, and 117 patients – to the control group.

Table 10. Rate of resolution of HHV infections symptoms against the therapy with PROTEFLAZID®

Author	Treatment group		Control group	
	Number of patients who were administered PROTEFLAZID® and who were observed disappearance of HHV infections symptoms	Total number of patients	Number of patients who were not administered PROTEFLAZID® and who were observed disappearance of HHV infections symptoms	Total number of patients
Gopchuk O.M., 2006	29	35	20	35
Zapolskyi M.E., 2012	31	32	16	26
Klimenko P.M., 2012	19	30	5	10
Total	79	97	41	71

On the indicator “Rate of disappearance of HHV infections symptoms during the PROTEFLAZID® administration”, 168 patients with HHV infections symptoms were examined, 97 of whom were included to the treatment group and 71 – to the control group.

Table 11. Rate of the positive treatment effect in patients with viral-bacterial diseases

Author	Treatment group		Control group	
	Number of patients who were administered PROTEFLAZID®, who showed positive dynamics	Total number of patients	Number of patients who were not administered PROTEFLAZID® and who showed positive dynamics	Total number of patients
Gopchuk O.M., 2006	29	35	20	35
Baev A.I., 2011	72	80	43	60
Total	101	115	63	95

On the indicator “Rate of positive dynamics to recovery in patients with viral-bacterial diseases”, 210 patients with viralbacterial diseases were examined, of whom 115 were included to the treatment group, and 95 – to the control group.

Discussion

Efficacy evaluation of the IMMUNOFLAZID® and FLAVOZID® administration in the treatment of viral and viral-bacterial diseases in children.

Table 12 shows sample statistics of the main immunological indicators of children with ARD, who were administered and were not administered the medications IMMUNOFLAZID® and FLAVOZID®.

Table 12. Sample statistics of the main immunological indicators of children with ARD, who were administered and were not administered the medications IMMUNOFLAZID® and FLAVOZID®

Parameter		Mean	№	Standard deviation	Standard error of the mean
Pair 1	Total CD3+ count in children with ARD who were administered IMMUNOFLAZID® and FLAVOZID®, %	59,9375	4	9,88799	4,94399
	Total CD3+ count in children with ARD who were not administered IMMUNOFLAZID® and FLAVOZID®, %	46,9250	4	6,66702	3,33351
Pair 2	Total CD4+ count in children with ARD who were administered IMMUNOFLAZID® and FLAVOZID®, %	39,0250	4	6,80361	3,40181
	Total CD4+ count in children with ARD who were not administered IMMUNOFLAZID® and FLAVOZID®, %	31,9000	4	8,08661	4,04331

Table 13 summarizes the results of testing the significance of the main immunological indicators of children with ARD who were administered and were not administered the medications IMMUNOFLAZID® and FLAVOZID®, using the Student’s t-test.

Table 13. Significance testing results of the main immunological indicators of children with ARD who were administered and were not administered IMMUNOFLAZID® and FLAVOZID®, using Student’s t-test

Parameter		Differences					t	Degrees of freedom	Two-tailed significance
		Mean	Standard deviation	Standard error of the mean	95% confidence interval for a difference between means				
					Lower bound	Upper bound			
Pair 1	Total CD3+ count in children with ARD who were administered IMMUNOFLAZID® and FLAVOZID® – Total CD3+ count in children with ARD who were not administered IMMUNOFLAZID® and FLAVOZID®	13,01250	5,85895	2,92947	3,68960	22,33540	4,442	3	0,021
Pair 2	Total CD4+ count in children with ARD who were administered IMMUNOFLAZID® and FLAVOZID® – Total CD4+ count in children with ARD who were not administered IMMUNOFLAZID® and FLAVOZID®	7,12500	2,19146	1,09573	3,63790	10,61210	6,503	3	0,007

The performed calculations confirmed the efficacy of the IMMUNOFLAZID® and FLAVOZID® administration in children with ARD in relation to the total CD3 + and CD4+ count, since the statistical significance of Student’s t-test is less than 0.05. The total CD3+ and CD4+ count in children with ARD who were administered IMMUNOFLAZID® and FLAVOZID® is within normal limits. The total CD3+ count in children with ARD who were administered IMMUNOFLAZID® and FLAVOZID® compared with children who were not administered these drugs increases on average by 27.7%. Similarly, the total CD4+ count in children with ARD who were administered IMMUNOFLAZID® and FLAVOZID® compared with children who were not administered these drugs increases on average by 22.3%. These changes have a positive effect on the immunity of children with ARD.

Figure 1 shows the forest plot of the meta-analysis results based on identifying the odds ratio of the clinical benefit rate of the drugs IMMUNOFLAZID® and FLAVOZID® administration in children with acute respiratory diseases in the treatment and control groups.

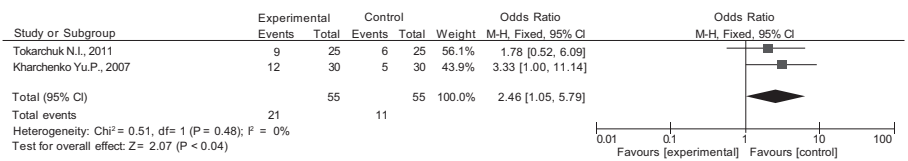


Figure 1. Meta-analysis results based on identifying the odds ratio of the clinical benefit rate of the drugs IMMUNOFLAZID® and FLAVOZID® administration in children with acute respiratory diseases

The χ -square value of the criterion ($P=0.48$) and I^2 -test ($I^2=0\%$) indicate the homogeneity of these trials, therefore, a model with a fixed effect was chosen. The P -value of the Fisher criterion ($P=0.04$) demonstrates the significance of selected effect.

The value of odds ratio ($OR=2.46$) indicates that probability of the clinical benefit rate of the drugs IMMUNOFLAZID® and FLAVOZID® administration in children with acute respiratory diseases in the treatment group is 2.46 times higher than in the control group.

The study provided by Tokarchuk N.I. (2011) represents the greatest weight, since the author determines the high efficacy of the drug IMMUNOFLAZID®. So, the IMMUNOFLAZID® administration helps to restore the functional status of the thymus (the thymulin level normalization), indicators of the T-cellular component of immune system, and the coefficients of nonspecific defense factors ($p<0.05$). The inclusion of IMMUNOFLAZID® in the treatment of ARD in young children reduces the duration of hospital stay ($p<0.05$) [15].

Efficacy evaluation of the PROTEFLAZID® administration in the treatment of viral and viral-bacterial diseases in adult patients

Table 14 shows a sample statistic of the main immunological indicators of patients with viral-bacterial diseases, who were administered and who were not administered PROTEFLAZID®.

Table 14. Sample statistic of the main immunological indicators of patients with viral-bacterial diseases, who were administered and who were not administered PROTEFLAZID®

Parameter		Mean	N	Standard deviation	Standard error of the mean
Pair 1	Total CD3+ count in patients with viral-bacterial diseases who were administered PROTEFLAZID®, %	67,0273	11	3,80913	1,14850
	Total CD3+ count in patients with viral-bacterial diseases who were not administered PROTEFLAZID®, %	59,5073	11	4,50884	1,35947
Pair 2	Total CD4+ count in patients with viral-bacterial diseases who were administered PROTEFLAZID®, %	43,4190	10	1,96431	0,62117
	Total CD4+ count in patients with viral-bacterial diseases who were not administered PROTEFLAZID®, %	37,7420	10	4,91400	1,55394
Pair 3	The value of CD4/CD8 in patients with viral-bacterial diseases who were administered PROTEFLAZID®, c.u.	2,1160	10	0,42516	0,13445
	The value of CD4/CD8 in patients with viral-bacterial diseases who were not administered PROTEFLAZID®, c.u.	1,7990	10	0,46127	0,14586

Table 15 summarizes the significance testing results of the main immunological indicators of patients with viral-bacterial diseases who were administered and were not administered PROTEFLAZID®, using the Student’s t-test.

Table 15. Significance test results of the main immunological indicators of patients with viral-bacterial diseases who were administered and were not administered PROTEFLAZID®, using Student’s t-test

Parameter		Differences					t	Degrees of freedom	Two-tailed significance
		Mean	Standard deviation	Standard error of the mean	95% confidence interval for a difference between means				
					Lower bound	Upper bound			
Pair 1	Total CD3+ count in patients with viral-bacterial diseases who were administered PROTEFLAZID® – Total CD3+ count in patients with viral-bacterial diseases who werenot administered PROTEFLAZID®	7,52000	5,49501	1,65681	3,82840	11,21160	4,539	10	0,001
Pair 2	Total CD4+ count in patients with viral-bacterial diseases who were administered PROTEFLAZID® – Total CD4+ count in patients with viral-bacterial diseases who werenot administered PROTEFLAZID®	5,67700	5,00113	1,58150	2,09941	9,25459	3,590	9	0,006
Pair 3	The value of CD4/CD8 in patients with viral-bacterial diseases who were administered PROTEFLAZID® – The value of CD4/CD8 in patients with viral-bacterial diseases who were not administered PROTEFLAZID®	0,31700	0,38690	0,12235	0,04023	0,59377	2,591	9	0,029

The performed calculations confirmed the efficacy of the Proteflazid® administration in patients with viral-bacterial diseases in relation to the total CD3 + and CD4+ count, since the statistical significance of Student’s t-test is less than 0.05. The total count of CD3+, CD4+ and CD4/CD8 in patients with viralbacterial diseases who were administered PROTEFLAZID® is within the normal range. The total CD3+ count in patients with viral-bacterial diseases who were administered PROTEFLAZID® compared with patients who were not administered this medication, on average, increases by 12.6%. The total CD4+ count in patients with viral-bacterial diseases who were administered PROTEFLAZID® compared with patients who were not administered this medication, on average, increases by 15.0%. The value CD4/CD8 in patients with viral-bacterial diseases who were

administered PROTEFLAZID® compared with patients who were not administered this medication, on average, increases by 17.6%. These changes have a positive effect on the immunity of patients with viral-bacterial diseases.

The forest plot, which can be seen in fig. 2, shows the results of meta-analysis based on the odds ratio of the relapse rate of genital herpes caused by HVI in the treatment and control groups.

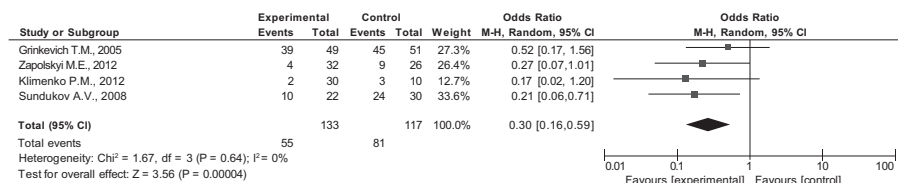


Figure 2. Results of meta-analysis based on the odds ratios of the relapse rate of genital herpes caused by HVI

The χ -square value of the criterion ($P=0.64$) and I^2 -test ($I^2=0\%$) indicate the homogeneity of these trials, therefore, a model with a fixed effect was chosen. The P-value of the Fisher criterion ($P=0.0004$) demonstrates the significance of selected effect.

The value of odds ratio ($OR=0.30$) indicates that probability of the relapse rate of genital herpes caused by HVI in the treatment group is 3.33 times lower than in the control one.

The study provided by Grinkevich T.M. (2005) has the greatest weight, due to which the high efficacy of the drug PROTEFLAZID® is determined [27]. In the study of A.V. Sundukov et al. (2008), a significant reduction in the disease duration and a decrease in the number of relapses were reported [35]. In the study provided by Klimenko P.M. et al. (2012), there was pointed out that the inclusion of PROTEFLAZID® in the treatment regimen made it possible to reduce the duration of genital herpes relapse in patients of the treatment group by 1.5 times compared with the control one [30].

The forest plot, which can be seen in fig. 3, shows the metaanalysis results based on the odds ratio of the rate of HVI symptom disappearance during the therapy with PROTEFLAZID® in the treatment and control groups.

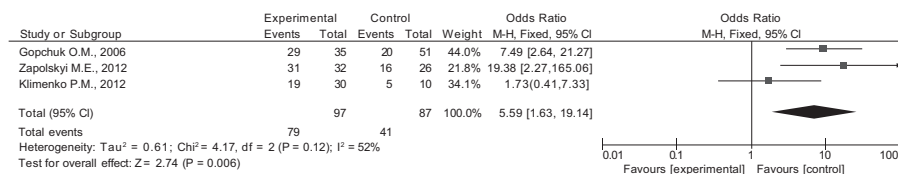


Figure 3. Meta-analysis results based on the odds ratio of the rate of HVI symptoms resolution during the therapy with PROTEFLAZID®

The χ -square value of the criterion ($P=0.12$) and I^2 -test ($I^2=52\%$) indicate the heterogeneity of these trials; therefore, a random effects model was chosen. The P-value of the Fisher criterion ($P=0.006$) demonstrates the significance of selected effect. The value of odds ratio

(OR=5.59) indicates that probability of the rate of HVI symptoms resolution during the therapy with PROTEFLAZID® in the treatment group is 5.59 times higher than in the control group.

The study conducted by O.M. Gopchuk (2006) has the greatest weight, because it indicated that the PROTEFLAZID® administration in the complex therapy of herpes virus lesions of the genitals in comparison with the routine methods is characterized by greater clinical, microbiological, and immunological efficacy [26]. It is noted in the study conducted by M.E. Zapolskyi (2012) that the use of PROTEFLAZID® promotes a significant CIC decrease ($p<0.05$) and increase in IgG [28].

Sensitivity analysis

The sensitivity analysis was conducted in such a way as to assess the effect of each particular trial on the integrated data, without taking into consideration case studies. The sensitivity analysis results showed that not a single case study significantly affected the integrated data, which indicates statistically significant results.

Assessment of publication bias

The funnel plot was used to assess the publication bias included in the study (fig. 4-6). Virtually all ES values in Figs 4-6 are within the funnel, which indicates unbiasedness.

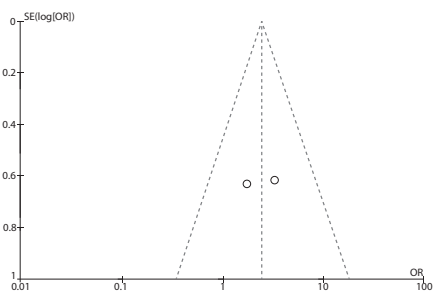


Figure 4. Funnelplot for the parameter “Clinical benefit rate of the IMMUNOFLAZID® and FLAVOZID® administration in children with acute respiratory diseases”

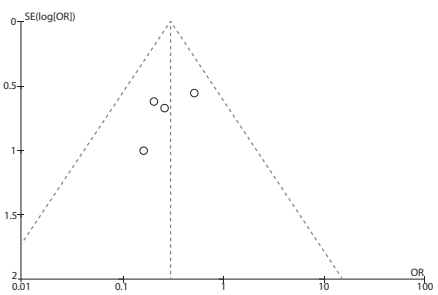


Figure 5. Funnelplot for the parameter “Relapse rate of genital herpes caused by HVI”

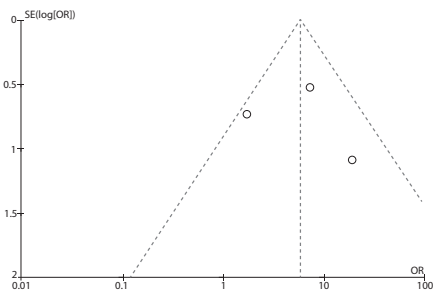


Figure 6. Funnelplot for the parameter “Rate of resolution of HVI symptoms during the therapy with PROTEFLAZID®”

Conclusions

The IMMUNOFLAZID® (FLAVOVIR®), FLAVOZID® administration in children with acute respiratory diseases in comparison with patients who were not administered these medications, increase the relative count of T-lymphocytes (CD3+) by 27.7% and N-helpers (CD4+) by 22.3%.

PROTEFLAZID® in the adult category of patients with viral-bacterial diseases in comparison with patients who were not administered this medication increases the relative count of T-lymphocytes (CD3+) by 12.6%, T-helpers (CD4+) – by 15.0%, and the immunoregulatory index (CD4/CD8) increases by 17.6%.

The meta-analysis revealed: the probability of the clinical efficacy rate when using the drugs IMMUNOFLAZID® (FLAVOVIR®) AND FLAVOZID® in children with acute respiratory diseases in the treatment group is 2.46 times higher than in the control; the probability of the relapse rate of genital herpes caused by HVI in the treatment group is 3.33 times lower than in the control one; the probability of the resolution of HVI symptoms during the therapy with PROTEFLAZID® in the treatment group is 5.59 times higher than in the control group.

The meta-analysis and analysis based on the Student's t-test indicate the high efficacy of flavonoids [PROTEFLAZID®, IMMUNOFLAZID® (FLAVOVIR®), FLAVOZID®] in the treatment of viral and viral-bacterial diseases in children and adults. The therapy using the abovementioned drugs helps to improve the clinical status and normalize the cellular immunity indicators in patients who received a treatment course.

References:

1. Баринський І. Ф. Герпесвірусні інфекції – імунodefіцитні захворювання ХХІ століття // Актуальні проблеми герпесвірусних інфекцій. – М., 2004. – С. 5–7.
2. Крамарьов С. О., Гриневич О. Й., Тонковід О. Б. [та ін] Метааналіз результатів клінічних досліджень ефективності флавоноїдів при вірусних та вірусно-бактеріальних захворюваннях у дітей// Сучасна педіатрія. – 2014. – №5 (61). – С. 1–7.
3. Медична мікробіологія, вірусологія та імунологія / За редакцією заслуженого діяча науки РФ, академіка РАН А. А. Воробйова. – Москва. – 2004. – С. 259–260.
4. Імунологія / За ред. Кузнецової Л. В., Бабаджана В. Д., Харченко Н. В. – Вінниця. – ТОВ «Меркьюрі-Поділля». – 2013. – 565 с.
5. Matyash V, Grynevych O, Broun T. Proteflazid®: догляд за herpesvirus and mixed infections. Meta-analysis of clinical trials results // Polski Merkuriusz Lekarski. – 2018. – Tom X88LIV. – №263. – P. 236–247.
6. Бекетова Г. В., Хайтович Н. В., Гриневич О. Й. Імунофлазид в педіатрії: системний аналіз ефективності та безпеки застосування // Педіатрія. Східна Європа. – 2014. – №3 (07). – С. 141–152.
7. Кузнецова Л. В. Вплив флавоноїдів на показники клітинного імунітету у дітей та підлітків, які хворіють на грип та гострі респіраторні вірусні інфекції, до та після лікування/Лабораторна діагностика. Східна Європа. – 2017. – Том 6. – №3. – С. 429–437.
8. Beniuk V., Kuznetsova L., Grynevych O., Broun T., Momotiuk L., Motuzka O., Yashchenko L. // Proteflazid®: outcome analysis of clinical trials studying the impact of human herpesvirus infection on some cellular immunity indicators and gestation course in women// Pol. Med. J., 2019-XLVI (274): 157–164.
9. Співак М. Я., Рибалко С. Л., Старосила Д. Б. та ін. Оцінка впливу флавоноїдвмісного препарату протекфлазид на моделі папіломавірусної інфекції *in vitro*/ Допов. Нац. акад. наук Укр. – 2018. – №10. – С. 103–111.

10. Matyash V., Grynevych O., Panasyuk O. [et all]. Proteflazid®: specific activity of herpes virus in pre-clinical investigations and its efficacy/safety in clinic practice (systematic review)//Yale Review of Education and Science. – 2015. – V.VI, №1 (16). – P. 422–462.
11. Beketova Beketova G., Grynevych O., Solomakha L., Golovnia N. Proteflazid®: Clinical experience in children of young and preschool age – systematic review of postmarketing surveillance // Polski Merkuriusz Lekarski. – 2018. – Tom XLIV. – №260. – P. 75–81.
12. Волосовець О. П. Оцінка ефективності та переносимості препарату «Флавозід» у дітей із гострою респіраторною вірусною інфекцією з розвитком обструктивного синдрому: Звіт, 2008. 25 с.
13. Крючко Т. О., Кінаш Ю. М. Оцінка ефективності Флавозиду у реабілітаційній терапії дітей молодшого віку з рецидивуючим бронхітом / Актуальні питання педіатрії, 2009. – №3 (39). – С. 1–5.
14. Кузнецова Л. В. Вплив флавоноїдів на показники клітинного імунітету у дітей та підлітків, які хворіють на грип та гострі респіраторні вірусні інфекції, до та після лікування // Лабораторна діагностика. Східна Європа. – 2017. – Том 6. – №3. – С. 429–436.
15. Токарчук Н. І., Старинець Л. С. Використання імунофлазиду для профілактики та лікування грипу та ГРВІ у дітей під час сезонного підвищення захворюваності // Сучасна педіатрія. 2012. – №1 (41). – С. 123–127.
16. Харченко Ю. Л. Застосування препарату Флавозід при інфекційному мононуклеозі у дітей // Сучасна педіатрія, 2007. – №4 (17). – С. 115–118.
17. Антонова Н. М., Савічук Н. О. Особливості медичного використання препарату Флавозід для лікування гострого герпесу порожнини рота та профілактики рецидивів герпетичної інфекції у дітей // Збірник наукових праць Національної медичної академії післядипломної освіти ім. П. Л. Шупика, 2010. – Вип. 19, книга 3. – С. 326–335.
18. Бережний В. В., Єршова І. Б., Кунегіна О. М. Гострі респіраторні вірусні захворювання у дітей та підлітків. – Київ; Луганськ, 2003. – С. 47–51.
19. Знам'янська Т. К. Вивчення ефективності та переносимості препарату «Флавозід» у новонароджених дітей від матерів з герпесною та цитомегавірусною інфекцією: Звіт. Інститут педіатрії, акушерства та гінекології АМН України, відділення неонатології, 2008. 25 с.
20. Єршова І. Б., Гончарова Т. А., Скородумова Н. П. Клініко-імунологічне обґрунтування застосування Лаферону та Протефлазиду при лікуванні серозних менінгітів у дітей // Проблеми екологічної та медичної генетики та клінічної імунології: збірник наукових праць, Київ; Луганськ, Харків, 2003. – С. 83–91.
21. Крючко Т. А., Несіна І. М., Кошлякова Є. С., Костейко Ю. А. Досвід застосування протефлазиду в комплексній терапії нейроінфекції у дітей // Імунологія та алергологія, 2002. – №3. – С. 60.
22. Шамсієв Ф. М., Мірсаліхова Н. Х., Алімова К. І., Таджиханова Д. П. Стан імунного статусу та ефективність протефлазиду у комплексній терапії дітей з бронхолегеневою патологією, асоційованою з TORCH інфекцією // Сучасна педіатрія, 2011. –2(36). – С. 39–41.
23. Юліш Є. І., Чернишова О. Є., Сорока Ю. Л. Тривалий субфебрилітет у дітей. Можливі причини та підходи до терапії // Сучасна педіатрія, 2011. – №1 (35). – С. 67–72.
24. Басв А. І., Алієв А. Т., Кузієва Г. Д. та ін. Обґрунтування застосування протефлазиду в комплексній терапії мікст-ІПСШ (повідомлення 1) // Питання дерматології та венерології, 2011. – №3–4 (47-48). – С. 24–37.
25. Башмаков М. М. Оцінка ефективності протефлазиду в комбінації з ентеросорбцією при імунореабілітації хворих на гнійно-некротичні ускладнення на тлі цукрового діабету в комплексі

- хірургічного лікування // Проблеми екологічної та медичної генетики та клінічної імунології: збірник наукових праць. – Київ; Луганськ; Харків, 2003. – С. 61–70.
26. Гопчук О. М. Корекція порушень менструального циклу у жінок із герпетичною інфекцією // Репродуктивне здоров'я жінки, 2006. – С. 187–190.
27. Грінкевич Т. М. Сучасні методи лікування генітального герпесу у жінок репродуктивного віку: Автореферат дис. канд. мед. наук: спец. Акушерство та гінекологія: 14.01.1/ Т. М. Грінкевич. – К., 2005. – 24 с.
28. Запольський М. Е. Багатоморфна ексудативна еритема, асоційована з герпесвірусом. епідеміологія та патогенетично обґрунтована // Терапія клінічна імунологія. алергологія. Інсектологія, 2012. – №8 (57). – С. 1–5.
29. Зелений І. І. Ефективність протефлазиду в імунореабілітації хворих на бешиху з гнійно-некротичними ускладненнями // Проблеми екологічної та медичної генетики та клінічної імунології: збірник наукових праць. – Київ; Луганськ; Харків, 2003. – С. 92–96.
30. Клименко П. М. Застосування препарату протефлазид для лікування хворих з урогенітальним герпесом / П. М. Клименко, Ю. Н. Гурженко, І. М. Лукман, В. А. Чабанов // Здоров'я чоловіка. – 2012. – №4. – С. 122–126.
31. Ковешніков О. В. Ефективність протефлазиду у комплексі медичної реабілітації хворих після лапароскопічної холецистектомії. – Київ; Луганськ; Харків, 2003. – С. 271–278.
32. Краснова С. П. Клініко-патогенетичне обґрунтування використання комбінації ербісолу та протефлазиду у лікуванні хворих з хронічною патологією органів травлення проблеми екологічної та медичної генетики та клінічної імунології, 2003. – Вип 1 (47). – С. 278–285.
33. Лещинський П. Т., Гусакивська О. В. Ефективність протефлазиду в комбінації з тимогеном при лікуванні жінок хворих на хронічний урогенітальний хламідіоз та динаміка імунологічних показників. – Київ; Луганськ; Харків, 2003. – С. 340–347.
34. Сімрок В. В., Удовіка Н. О. Вплив протефлазиду на клініко-імунологічні показники у комплексі реабілітації жінок, які перенесли загострення хронічного сальпінгоофориту // Вісник акушерів-гінекологів України, 2002. – №5–6 (20). – С. 51–55.
35. Скриньок А. В. Про результати клінічних випробувань препарату ПРОТЕФЛАЗИД у терапії герпесвірусних інфекцій [Звіт]. – Москва, 2008. – С. 16.
36. Удовіка Н. О. Вплив протефлазиду на клітинні показники імунітету у жінок, хворих на хронічні сальпінгофори з наявністю синдрому підвищеної стомлюваності // Проблеми екологічної та медичної генетики та клінічної імунології: збірник наукових праць. Київ; Луганськ; Харків, 2003. – С. 363–376.
37. Соцька Я. А. Ефективність ПРОТЕФЛАЗИДУ у медичній реабілітації хворих на хронічний бронхіт на тлі повторних ангіні // Збірник наукових робіт. – Луганськ. – 2003. – 4 с.
38. Хрущ В. І. Аспекти патогенетичної терапії інфекційної, екзема та атопічного дерматиту // Клінічна імунологія. алергологія. Інсектологія, 2016. – Спецвипуск №1. – С. 60–65.
39. Гошко О. Л., Рибалко С. Л., Матяш В. І., Атаманюк В. П. Вивчення інтерфероногенної та імуномодулюючої активності протефлазиду у хворих з герпесвірусним ураженням нервової системи // Лабораторна діагностика, 2005. – №4 (34). – С. 30–35.
40. Гурженко Ю. М. Зміни лейкограми, імунологічних показників крові та ревмопроб при курсовому лікуванні хворих на хворобу Пейроні // Здоров'я чоловіка, 2003. – 3 (6). – С. 49–53.
41. Гурженко Ю. М. Імунотерапія запальних захворювань чоловічих статевих органів з використанням препарату ПРОТЕФЛАЗИД // Здоров'я чоловіка, 2005. – №1 (12). – С. 83–86.

42. Гурженко Ю. М., Клименко П. М., Федорук О. С. Терапія запальних захворювань чоловічих статевих органів хламідійної етіології з використанням препарату ПРОТЕФЛАЗІД // Здоров'я чоловіка, 2010. – 4 (35). – С. 48–51.
43. Курбацька О. В. Ефективність протепфлазиду у комплексі медичної реабілітації жінок з непліддям імунного генезу. – Київ; Луганськ; Харків, 2003. – С. 333–340.
44. Лоскутова І. В. Ефективність нових протівірусних препаратів – амізону та ПРОТЕФЛАЗІДУ у лікуванні хворих з тяжкою формою епідемічного паротиту // Проблеми екологічної та медичної генетики та клінічної імунології: збірник наукових праць. – Київ; Луганськ; Харків, 2003. – С. 117-126.
45. Панасюк О. Л. Етіопатогенетична терапія герпесвірусної інфекції із застосуванням протепфлазиду. – 14.01.13 – інфекційні хвороби. – К., 2007.
46. Сергієнко С. В., Петруня О. М. Ефективність застосування протепфлазиду при лікуванні хворих на посттравматичні увеїти. // Проблеми екологічної та медичної генетики та клінічної імунології: збірник наукових праць. – Київ; Луганськ; Харків, 2003. – С. 323–333.
47. Сидоренко О. В. Фактори міжклітинної кооперації імунної системи та можливості імунокорекції у хворих на хронічний пієлонефрит: Автореферат дис. канд. мед. наук: спец. Імунологія та алергологія: 14.03.08 / О. В. Сидоренко. – Київ, 2010. – 24 с.
48. Танько О. П., Самойлова М. В. Імунотерапія запальних процесів геніталій у пубертатному віці // Здоров'я жінки, 2008. – №1 (33). – С. 104–106.
49. Танько О. П., Скорбач Ю. І. Імуномодуюча терапія вульвовагінітів у вагітних // Репродуктивне здоров'я жінки, 2007. – №1 (30). – С. 90–92.
50. Шаповалова І. А. Застосування протепфлазиду в комплексній терапії та імунореабілітації підлітків з ангінами змішаної вірусно-бактеріальної етіології // Проблеми екологічної та медичної генетики та клінічної імунології: збірник наукових праць. – Київ; Луганськ; Харків, 2003. – С.1–6.

Reference: Beketova G., Kuznetsova L., Grynevych O., Broun T., Momotiuk L., Motuzka O., Yashchenko L. (2021) Analysis of the clinical trial results of the flavonoid effects on some cellular immunity indicators in children and adults. *Pol Med J., XLIX (289): 44–53.*

1.8. PROTEFLAZID® VS COVID-19: SUCCESSES IN RESEARCH

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Abstract

The original antiviral drug PROTEFLAZID® has been used in clinical practice since the early 2000s for the etiotropic treatment of acute respiratory viral diseases due to its ability to block polymerases of RNA and DNA viruses. Given that at the beginning of the global pandemic COVID-19 caused by RNA-containing virus of the species SARS-CoV-2 in 2020, the ability of the active substance of the drug to inhibit the activity of 3CL-protease coronavirus SARS-CoV-2 and subsequently confirm the property of the active substance to block the replication of SARS-CoV-2 virus in cell cultures *in vitro*, it has been extremely important to test the effectiveness of the drug PROTEFLAZID®, drops for the prevention and treatment of COVID-19 in «off label use».

In retrospective clinical studies, it has been shown that PROTEFLAZID® in the conditions of the COVID-19 pandemic contributes to a 2.2-fold reduction in the proportion of sick medical staff; reduction in the proportion of patients with severe disease by 3.3 times; reduction of the average duration of treatment by 1.8 times; and the most important is that there is no mortality among the sick medical staff and patients who has administered the drug.

Key words: PROTEFLAZID®, 3CL protease, RNA polymerase, SARS-CoV-2, COVID-19.

Introduction

By the early 2000s, coronavirus infection was already recognised as a growing concern. A 2013 publication titled “Coronavirus: Problems and Prospects” underscored the potential threat this infection posed to global public health [1].

The medicinal product PROTEFLAZID® is a herbal ethanol extract from green parts of cereal grasses of Gramineae family – Calamagrostis Adans and Deschampsia Beauv, which contains biologically active substances with polypharmacological action. The active substance of PROTEFLAZID® API consists of flavonoid aglycones: tricrin, apigenin, and luteolin. The pharmacologically active molecules of PROTEFLAZID® API are flavones and flavonoids that form the following complexes: [tricrin, tricrin-7-O- or 8-C glycoside] : [luteolin-7-O- or 8-C glycoside]: [apigenin, apigenin-7-O-glycoside or 8-C glycoside], within a “matrix” of excipients – amino acids; carboxylic acids and sugars; polysaccharides, pectins, chlorophylls (a and c), and hemicelluloses (A and B). Pharmaceutical

form: drops; pharmacotherapeutic group: direct-acting antiviral agents; ATC code: J05A X [2–4].

Specific Antiviral Activity of PROTEFLAZID® Against RNA-Containing Coronaviruses, Including SARS-CoV-2: Evidence from Recent Preclinical Studies

The potential of PROTEFLAZID® to inhibit the replication of both RNA and DNA viruses, through the suppression of RNA and DNA polymerases, was first established 15 years ago. This property provided the theoretical rationale for initiating preclinical studies aimed at evaluating the antiviral potential of the product's active substance against coronaviruses [5–7].

RNA polymerase, 3CL protease, and transcription factor Nrf2 are recognised as primary targets for antiviral drugs against SARS-CoV-2. According to data published in early 2020 by both international and Ukrainian researchers, flavonoids inhibit 3CL protease activity and bind angiotensin-converting enzyme 2 (ACE2) – a receptor for the coronavirus spike glycoprotein. Furthermore, activation of the transcription factor Nrf2 contributes to the reduction of oxidative stress and inflammation [8–26].

In early 2020, molecular docking studies demonstrated that the ligand fits well within the catalytic pocket of the SARS-CoV-2 3CL protease, forming hydrogen bonds with the Ser144 and Gly143 residues, and positioning itself between the catalytic residues His163, Asn142, and Cys145. Additionally, it establishes hydrogen bonds with two catechol hydroxyl groups with the Glu166 residue, indicating potential inhibition of SARS-CoV-2 3CL protease activity [6, 9].

A study at Saarland University (Germany) assessed the targeted inhibition of SARS-CoV-2 3CL protease activity by PROTEFLAZID® and showed that PROTEFLAZID® has a strong potential to inhibit the SARS-CoV-2 3CL protease [1, 27].

In experimental research by the SI “L. V. Hromashevskyi Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine,” PROTEFLAZID® demonstrated potent antiviral activity in vitro against porcine transmissible gastroenteritis coronavirus (TGEV). The drug inhibits replication of RNA-containing virus (efficacy confirmed in two cell lines; viral load reduced by six logarithmic units), which is structurally similar to human coronaviruses [6].

The findings of Ukrainian researchers were validated on the models of human respiratory coronavirus HCoV-229E at the Division of Experimental Virology, Hannover Medical School (Germany), and SARS-CoV-2 at the CReSA BSL3 laboratory of the Animal Health Research Center, the Autonomous University of Barcelona (Spain), using a SARS-CoV-2 isolate of severe acute respiratory syndrome. Additional validation was performed at the Galveston National Laboratory (USA).

The antiviral activity of PROTEFLAZID® was evaluated in a passaged monolayer culture of Vero E6 cells (ATCC CRL-1586) using a quantitative luminescent bio-analytical assay based on a dual-luciferase reporter system. To eliminate nonspecific cytotoxic effects of the alcohol solvent on Vero E6 cells during assays with human respiratory coronavirus HCoV-229E (Germany), the alcohol was removed by evaporation, and the resulting dry residue was stabilised with 1% DMSO. Quantitative and qualitative analysis of the formulation following alcohol removal was performed using high-resolution liquid chromatography coupled with mass spectrometry (LC-MS).

A dilution series of the alcohol-free PROTEFLAZID® substance (5.3–0.1 µg/mL) demonstrated that at a concentration of 1.0 µg/mL, the replication of HCoV-229E coronavirus was inhibited by 92% without cytotoxic effects on Vero E6 cell cultures. Increasing the concentration to 5.3 µg/mL resulted in 100% inhibition of HCoV-229E virus replication [27].

The specific antiviral activity of PROTEFLAZID® against SARS-CoV-2 coronavirus was evaluated *in vitro* at the Animal Health Research Center (IRTA-CReSA, the Autonomous University of Barcelona, Spain) using Vero E6 cell cultures. The medicinal product PROTEFLAZID® (drops), in a series of dilutions ranging from 0.0000819 µg to 160 µg, was added to Vero E6 cell cultures simultaneously infected with SARS-CoV-2 virus at an infectious dose of TCID₅₀/mL 101.8, followed by incubation. Viral infection of the cell culture was evaluated microscopically based on the cytotoxic effects on cells. PROTEFLAZID® exhibited potent antiviral activity against SARS-CoV-2 virus at concentrations ranging from 1.28 to 6.4 µg [33].

In vitro studies using the African green monkey kidney cell line (Vero E6) and ACE2-expressing human lung adenocarcinoma cell line (A549/ACE2) conducted by the Galveston National Laboratory specialised in SARS/MERS/COVID-19 research (Department of Microbiology and Immunology, Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, USA) demonstrated that PROTEFLAZID® effectively prevents the virus-induced cytopathic effect caused by SARS-CoV-2 (USA-WA-1/2020 isolate) and provides complete (100%) antiviral protection against SARS-CoV-2-induced cellular damage [28].

Findings from international laboratories indicate that flavonoids (apigenin, luteolin, and quercetin) activate the transcription factor Nrf2, resulting in downregulation of angiotensin-converting enzyme 2 (ACE2) expression in respiratory epithelial cells. This reduction limits viral entry into epithelial cells and interferes with SARS-CoV-2 replication. Furthermore, activation of transcription factor Nrf2 helps prevent or reduce the severity of SARS-CoV-2 infection by protecting host cells from oxidative stress and inflammation, thereby reducing the risk of acute respiratory distress syndrome (ARDS) [12, 15, 17, 20–22].

Excessive production of reactive oxygen species (ROS), in conjunction with oxidative stress during severe COVID-19, is known to trigger a cytokine storm. In this setting, pro-inflammatory cytokines and ROS act synergistically to activate epithelial and endothelial cells of the lungs, leading to increased intercellular permeability in the lungs, which facilitates the leakage of exudate into the alveoli, thereby significantly impairing pulmonary gas exchange. Furthermore, the combined effects of the cytokine storm and oxidative stress induce ROS-dependent apoptosis of endothelial cells, contributing to the release of coagulation factors and the subsequent thrombosis.

The company, in collaboration with its research partners, is currently investigating the effects of PROTEFLAZID® on the transcription factor Nrf2. The findings will be shared in forthcoming scientific publications.

PROTEFLAZID® (drops) is a direct-acting antiviral agent. Its mechanism of action in COVID-19 acute respiratory viral infection is due to the following:

a) **blocking the activity of 3C-like protease**, which is vital for SARS-CoV-2 virus entry and proliferation;

b) ***blocking the RNA polymerase***, which is essential for SARS-CoV-2 virus replication.

Based on the preclinical data, SI “L.V. Hromashevskyi Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine,” the Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine, and UKRMEDPATENTINFORM at the Ministry of Health of Ukraine published and distributed among local and regional health authorities across Ukraine the Information bulletin, “ANTIVIRAL ACTIVITY OF THE FLAVONOID-BASED DRUG PROTEFLAZID®” [29].

Preclinical reports on the specific antiviral activity of PROTEFLAZID® in SARS-CoV-2 have been compiled as an annex to the registration dossier of PROTEFLAZID® (drops) and submitted for expert examination to the Ministry of Health of Ukraine. By Order No.1680 dated 6 August 2021, the Pharmacodynamics section of the PROTEFLAZID® (drops) prescribing information was modified as follows (underlined text):

“It has been demonstrated that the drug’s direct antiviral effect is due to inhibition of virus-specific enzymes – DNA and RNA polymerases, thymidine kinase, reverse transcriptase, 3CL protease, and neuraminidase.

PROTEFLAZID® inhibits the activity of the SARS-CoV-2 3CL protease, as demonstrated through molecular docking studies and confirmed using a 3CL protease MBP-tagged (maltose-binding protein fused to SARS-CoV-2) assay kit.

A dual luciferase reporter gene assay with Renilla (reflecting the replication of seasonal coronavirus CoV-229E) showed that the drug blocks viral replication.

In preclinical in vitro studies on monkey (Vero E6) and human (A549/ACE2) cell lines, PROTEFLAZID® demonstrated antiviral activity against the pandemic human coronavirus SARS-CoV-2, showing strong inhibition of viral replication” [4].

By Order of the Ministry of Health of Ukraine No.1922 dated 10 September 2021, similar updates were made to the prescribing information for FLAVOVIR® (syrup). This update is especially important given expert predictions that SARS-CoV-2 and its variants (mutated forms) posed a particular risk to children during the 2021–2022 epidemic season.

Retrospective Pilot Clinical Observations

In February 2020, before the WHO officially declared the pandemic, the pharmaceutical company “Ecopharm” launched a humanitarian initiative to protect healthcare professionals in clinics treating patients infected with SARS-CoV-2. As part of this effort, “Ecopharm” donated over 30,000 bottles of PROTEFLAZID® (drops) to more than 140 hospitals involved in COVID-19 patient care.

The scale of this charitable initiative, along with reports received from healthcare centres, made it possible to consolidate experience on PROTEFLAZID® administration for disease prevention and treatment. The National Academy of Statistics, Accounting and Audit analysed written reports of 79 healthcare centres across different regions of Ukraine on PROTEFLAZID® use. Healthcare professionals and patients started treatment with this medicinal product on 27 February 2020. The latest available information is as of 1 October 2020 [31].

The majority of reports from healthcare centres indicated that healthcare professionals had frequent contact with patients potentially infected with COVID-19 and were at high risk of infection.

During the period mentioned above, PROTEFLAZID® was administered to 8,572 persons for disease prevention, including 7,444 healthcare professionals and 1,128 patients, and to 433 persons for disease treatment. In total, 9,005 healthcare professionals and patients used PROTEFLAZID® (drops) for prevention and treatment. The statistical value for the “Number of fatalities” among the participating healthcare centres was “0”.

Compared to official data on COVID-19 outcomes, the cohort treated with PROTEFLAZID® demonstrated zero mortality, a 2.2-fold reduction in the proportion of infected healthcare professionals, a 3.3-fold decrease in the incidence of severe disease among patients, and a 1.8-fold reduction in average treatment duration [2, 31].

It is reasonable to suggest that COVID-19 morbidity and mortality rates among healthcare professionals and patients in Ukraine could have been significantly reduced through the timely and widespread use of PROTEFLAZID® during the outpatient stage.

Following a comprehensive review of preclinical data and clinical observations, experts from the National Academy of Medical Sciences of Ukraine have recommended the use of PROTEFLAZID® (drops) for both prophylactic and antiviral treatment in SARS-CoV-2 infection [32].

Drawing on evidence from preclinical studies, clinical experience, and the recommendations outlined in the Information bulletin “Antiviral Activity of the Flavonoid-Based Drug PROTEFLAZID®”, healthcare centres initiated the development of local clinical treatment protocols for primary care – “Coronavirus Disease (COVID-19). Outpatient Stage.” These protocols recommend the use of PROTEFLAZID® (drops) in patients with mild to moderate disease in outpatient settings. Early administration of direct-acting antiviral agents is critical to prevent a dangerous rise in viral load in infected individuals. According to the protocol developers, this strategy could significantly ease the burden of inpatient hospital services.

Nevertheless, the absence of recommendations for early outpatient etiotropic treatment in all current editions of the Ministry of Health of Ukraine’s approved “Clinical Protocol for the Treatment of COVID-19 Coronavirus Disease” remains a matter of concern for the authors and many other healthcare professionals. This omission likely contributes to the substantial overburdening of inpatient healthcare facilities across the country.

Therefore, it is essential, particularly for general practitioners, to understand that COVID-19 is a systemic disease driven by the uncontrolled replication of SARS-CoV-2, leading to progressively increasing viral load and severe complications that vary depending on the disease stage.

Conclusions

COVID-19 belongs to acute respiratory viral infections (ARVIs) requiring prompt outpatient treatment immediately at the onset of symptoms. The earlier the treatment is initiated,

the better the outcome for the patient is likely to be. Early administration of an etiotropic antiviral agent can help prevent the rise in viral load and reduce the risk of complications associated with COVID-19 progression.

The medicinal product PROTEFLAZID® (drops) is an etiotropic agent that inhibits viral RNA polymerase and 3CL protease, as stated in the current approved prescribing information, indicated for the treatment of acute respiratory viral infections (ARVIs). Therefore, both from a legal and clinical standpoint, this medicinal product has a valid basis for use in the treatment of ARVIs caused by the SARS-CoV-2 virus.

References:

1. Abian O., Ortega-Alarcon D., Jimenez-Alesanco A. et al. Structural stability of SARS-CoV-2 3CLpro and identification of quercetin as an inhibitor by experimental screening. *International Journal of Biological Macromolecules* 2020. – Vol.164. – P. 1693–1703.
2. Дерябін О. Н., Завелевич М. П., Старосила Д. Б. та співавт. Природні поліфеноли як інгібітори взаємодії коронавірусів із клітинами: огляд літератури та експериментальні дані // *Укр.мед. часопис*. – 2020. – №3 (137), Т.1. С.1–5.
3. Chen S., Short J. A., Young D. F., Killip M. J., Schneider M., Goodbourn S., Randall R. E. Heterocellular induction of interferon by negative-sense RNA viruses. *Virology* 2010, 407, 247–255.
4. Chien-Te Kent Tseng, Dr. Aleksandra K. Drelich. Report «The efficacy assessment of new compound against SARS-CoV-2 infection in *in vitro* models. – Galveston National Laboratory, Galveston, USA.– 2021.– p. 4.
5. Grynevych O., Borshov S., Matyash V., Momotiuk L., Motuzka O., Yashchenko L. Proteflazid® effectiveness for prevention and treatment of acute viral respiratory infections in the conditions of COVID-19 in the conditions of SARS-COV-2 // *Pol. Med. J.*, 2021; XLIX (292); 255–265.
6. Гриневич О. Й., Маркович І. Г. Коронавірус: проблеми та перспективи // *Український медичний часопис*. – 2013. – №3. – С. 45–48.
7. Інструкція для медичного застосування лікарського засобу ПРОТЕФЛАЗІД® (Зміни внесено. Наказ МОЗУ №1680 від 06.08.2021 року).– 5 с.
8. Інформаційний лист про нововведення в сфері охорони здоров'я. Напрямок впровадження: віруси та вірусні інфекції. Антивірусна активність флавоноїдного препарату ПРОТЕФЛАЗІД. Укрмедпатентінформ, №80-2021.– 4 с.
9. Jo S., Kim S., Shin D. H., Kim M. S.. Inhibition of SARS-CoV 3CL protease by flavonoids. *J Enzyme Inhib Med Chem*. 2020; 35(1):145–151.
10. Karamuska T. Interim report «Evaluation of the antiviral activity of a test item against SARS-CoV-2 *in vitro* in Vero E6 cells» IRTA-CreSA Universitat Autònoma de Barcelona, Barcelona. Spain.– 2020 – p.8.
11. Lee C. Therapeutic Modulation of Virus-Induced Oxidative Stress via the Nrf2-Dependent Antioxidative Pathway. *Oxidative Med. Cell. Longev*. 2018, 2018, 6208067.
12. Li F. Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits. *J. Virol*. 2012, 86, 2856–2858.
13. Li W., Moore M. J., Vasilieva N., Sui J., Wong S. K., Berne M. A., Somasundaran M., Sullivan J. L., Luzuriaga K., Greenough T. C. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 2003, 426, 450–454.

14. Li F. Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits. *J. Virol.* 2012, 86, 2856–2858.
15. Luzhetskyy A. Report: Inhibition of 3CL protease (SARS-CoV-2) by proteflazid. – Helmholtz Centre for Infection Research. University of Saarland, Germany.– Saarbrücken. – 2021. – p.2.
16. Luzhetskyy A. Report on the activity of Proteflazidum against human coronavirus CoV229E. – Helmholtz Centre for Infection Research. University of Saarland, Germany. – Saarbrücken. – 2020. – p.2.
17. Matyash V., Grynevych O., Broun T. PROTEFLAZID®: clinical studies as evidence base of its antiviral activity // *Pol Med J.* 2019; XLVI (271); 30–31.
18. McCord J. M., Hybertson B. M., Cota-Gomez A., Gao B. Nrf2 Activator PB125® as a Potential Therapeutic Agent Against COVID-19. *bioRxiv* 2020.
19. Момотюк Л. Є., Ященко Л. О., Мотузка О. М. Заключний звіт «Формування бази даних та статистичний аналіз ефективності використання препарату ПРОТЕФЛАЗІД, краплі в умовах COVID-19». – Національна академія статистики, обліку та аудиту. – Київ. – 2020. – 38 с.
20. Nguyen T. T., Woo H. J., Kang H. K., Nguyen V. D., Kim Y. M., Kim D.тW., Ahn S. A., Xia Y., Kim D. Flavonoid-mediated inhibition of SARS coronavirus 3C-like protease expressed in *Pichia pastoris*. *Biotechnol. Lett.* 2012, 34, 831–838.
21. Patricia Mendonca and Karam F. A. Soliman. Flavonoids Activation of the Transcription Factor Nrf2 as a Hypothesis Approach for the Prevention and Modulation of SARS-CoV-2 Infection Severity – *Antioxidants* 2020, 9, 659; doi:10.3390/antiox9080659.
22. Пальчиовська Л. Г., Васильченко О. В., Платонов М. О. та співавт. Антивірусні властивості рослинних флавоноїдів – інгібіторів синтезу ДНК і РНК // *Biopolymers and Cell.* – 2013. – V.29, (2). – P.150–156.
23. Ryu Y. B., Jeong H. J., Kim J. H., Kim Y. M., Park J. Y., Kim D., Nguyen T. T., Park S. J., Chang J. S., Park K. H. et al. Biflavonoids from *Torreya nucifera* displaying SARS-CoV 3CL(pro) inhibition. *Bioorg. Med. Chem.* 2010, 18, 7940–7947.
24. Рекомендаційний лист НАМУ №5-03/487 від 16.03.2021 щодо доцільності використання препарату ПРОТЕФЛАЗІД®, краплі при COVID-19.– 1 с.
25. Рибалко С. Л. «Вивчення антивірусної активності препарату ПРОТЕФЛАЗІД®, його активних структур на моделі коронавірусу трансмісивного гастроентериту свиней» ДУ «Інститут епідеміології та інфекційних хвороб ім. Л. В. Громашевського Національної академії наук України», березень 2020 року. – 42 с.
26. Рибалко С. Л. «Звіт про вивчення механізмів дії біологічно-активних речовин лікувальної субстанції Протефлазіда. – Інститут епідеміології та інфекційних хвороб ім. Л. В. Громашевського НАМН України. – м. Київ. – 2010. – 83 с.
27. Seelinger G., Merfort I., Schempp C. M. Anti-oxidant, antiinflammatory and anti-allergic activities of luteolin. *Planta Med.* 2008;74(14):1667–1677.
28. Silva-Palacios A., Ostolga-Chavarria M., Zazueta C., Konigsberg M. Nrf2: Molecular and epigenetic regulation during aging. *Ageing Res. Rev.* 2018, 47, 31–40.
29. Theoharides T. C. COVID-19, pulmonary mast cells, cytokine storms and beneficial actions of luteolin. *BioFactors.* 2020; 1–3.
30. Wang M. M., Lu M., Zhang C. L., Wu X., Chen J. X., Lv W. W., Sun T., Qiu H., Huang S. H. Oxidative stress modulates the expression of toll-like receptor 3 during respiratory syncytial virus infection in human lung epithelial A549 cells. *Mol. Med. Rep.* 2018, 18, 1867–1877.

31. Weng Z., Patel A. B., Panagiotidou S., Theoharides T. C. The novel flavone tetramethoxyluteolin is a potent inhibitor of human mast cells. *J Allergy Clin Immunol.* 2015; 135(4):1044–52.e5.
32. Wrapp D., Wang N., Corbett K. S., Goldsmith J. A., Hsieh C.-L., Abiona O., Graham B. S., McLellan J. S. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020, 367, 1260–1263.
33. Xue G., Gong L., Yuan C., et al. A structural mechanism of flavonoids in inhibiting serine proteases. *Food Funct.* 2017; 8(7):2437–2443.
34. Zakaryan H., Arabyan E., Oo A., Zandi K. Flavonoids: Promising natural compounds against viral infections. *Arch. Virol.* 2017, 162, 2539–2551.

1.9. PROTEFLAZID® EFFECTIVENESS FOR PREVENTION AND TREATMENT OF ACUTE VIRAL RESPIRATORY INFECTIONS IN THE CONDITIONS OF COVID-19

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Abstract

The original antiviral drug PROTEFLAZID® has been used in clinical practice since the early 2000s for the etiotropic treatment of acute respiratory viral diseases, due to its property of blocking viral RNA and DNA polymerases. Considering that at the beginning of the global COVID-19 pandemic, caused by RNA-containing virus of SARS-CoV-2 species in 2020, the ability of the drug active substance to inhibit the activity of 3CL-protease of the SARS-CoV-2 coronavirus was shown by molecular docking and, subsequently, to confirm the property of the active substance to block the reproduction of the SARS-CoV-2 virus in cell cultures *in vitro*. It was extremely important to test the effectiveness of the drug PROTEFLAZID®, drops for the prevention and treatment of COVID-19 disease at «off labell use».

The aim of the study was to provide a statistical assessment of the effectiveness of the drug PROTEFLAZID®, drops in terms of COVID-19 pandemic.

Materials and methods. The analysis has been performed including the letters-references from medical institutions from different regions of Ukraine about the effectiveness of the drug PROTEFLAZID®, drops. Methods of statistical analysis have been focused on dynamics and structure analysis, meta-analysis, generalization, etc.

Results. 90 letters-references about the effectiveness of the drug PROTEFLAZID®, drops, during the prevention and treatment of acute respiratory viral infections, including COVID-19 diseases were analyzed. The study used references that contained the most complete information. The number of deleted letters-references is 11. Lettersreferences from 79 medical institutions from different cities and

regions of Ukraine were analyzed. The period of starting taking the drug by employees and patients of medical institutions began on February 27, 2020. Final information – October 01, 2020. The vast majority of letters from medical institutions indicated that medical staff were in contact with patients potentially suffering from COVID-19. This means a high risk of being infected with Coronavirus infection. Total number of patients who took PROTEFLAZID® for preventive purpose was 8,572, including 7,444 medical workers and 1,128 ordinary patients. Indicator «Number of fatalities» for the medical institutions providing such information was «0». Total number of patients who took PROTEFLAZID® for therapeutic purposes was 433, including 23 medical workers and 410 ordinary patients. Indicator «Number of fatalities» for the medical institutions providing such information was «0». The total number of medical personnel and patients who used PROTEFLAZID® drops for preventive and therapeutic purposes was 9005 people.

Conclusions. The statistical analysis confirmed the effectiveness of the drug PROTEFLAZID® for the prevention and treatment of COVID-19, as, when compared with official actual data, regarding the main indicators of the incidence of COVID-19: there were no fatalities; the average treatment period decreased (1.8 times); the proportion of recovered increased (at least 1.5 times); the proportion of sick medical workers in the total population of sick medical workers decreased (2.2 times); the proportion of patients with a severe course decreased (3.3 times). It can be argued that the drug PROTEFLAZID®, drops has shown high effectiveness in the prevention and treatment of acute respiratory viral infections, including COVID-19, among medical personnel and patients.

Key words: PROTEFLAZID®, SARS-CoV-2, «off-label use», COVID-19 prevention and treatment.

Introduction

In the early 2000s, the arsenal of antiviral agents was supplemented by the original drug Proteflazid® (Developed by "SMC "Ecopharm" Ltd., Kyiv, Ukraine) having direct antiviral, interferonogenic, immunomodulatory, antioxidant apoptosis – modulating effect, being of great practical importance, and accompanied by evidence-based scientific studies for the assessment of its effectiveness and safety in various pathologies of viral etiology.

Proteflazide® is an ethanol extract from herbal raw materials – green parts of wild cereal plants of Gramineae family, genera Calamagrostis Adans and Deschampsia Beauv, containing biologically active substances with polypharmacological action (PCTWO2004/058285; PCT/UA2015/000101). The active ingredients of Proteflazid API are flavonoid aglycon compounds: tricetin, apigenin, luteolin. The active molecules of the PROTEFLAZID The active molecules of API Proteflazid are flavones and flavone glycosides forming complexes of the following composition: [Tricetin, Tricetin-7-O or 8-C glycoside] : [Luteolin-7-O or 8-C glycoside] : [Apigenin, Apigenin-7-O glycoside or 8-C glycoside] in the "matrix" of excipients: amino acids; carboxylic acids and sugars; polysaccharides, pectins, chlorophyll (a and b) and hemicellulose (A and B).

More than 22 thousand people took part in clinical studies; among them, more than 12 thousand patients of various age, social and other groups took the drug Proteflazid® for etiotropic treatment of viral diseases caused by RNA – or DNA-containing viruses.

In order to confirm and summarize the scientific data obtained clinically, and to form an evidence base confirming the effectiveness and safety of the drug Proteflazid® in the period 2014-2018, a number of thematic meta-analyses and systematic reviews of clinical observations and long-term results of the drug use were carried out, which was reflected in scientific publications (meta-analyses and systematic reviews) [1-17].

Clinical studies have established and statistically confirmed that Proteflazid® is therapeutically effective at any stage of infection, and not only at the time of replication of the viral pathogen, unlike, for example, acyclic nucleosides. In addition, repeated and long-term courses of administering the drug did neither cause the development of resistant strains of viruses, nor lead to immunosuppression.

With regard to RNA-containing influenza viruses, Proteflazid® has been found to not only inhibit neuraminidase activity but, unlike other drugs in this class recommended for treatment of influenza, it also inhibited intracellular replication of influenza viruses by inhibiting the synthesis of virus-specific enzymes, namely thymidine kinase and RNA polymerase. Another significant difference between the drug Proteflazid® and influenza virus neuraminidase inhibitors (especially in epidemic periods, as in the structure of influenza morbidity is only 6 to 10% of ARVI patients) is that Proteflazid®, due to its direct antiviral activity against other causative agents of acute respiratory viral infections (adeno-, enteroviruses and others) can inhibit their replication as well.

Modern preclinical studies examine the specific antiviral activity of the active substance Proteflazid against RNA-containing coronaviruses, including SARS-CoV-2.

The property of the active ingredient Proteflazid to block the reproduction of RNA and DNA-containing viruses (through inhibition of RNA and DNA polymerase) was established 15 years ago and formed the basis for the theoretical justification of the attempt to investigate the active ingredient of the drug for its possible antiviral activity against coronaviruses in preclinical studies, as scientists worldwide are now actively searching for drugs – RNA polymerase and 3CL protease inhibitors of SARS-CoV-2 virus.

It is known that active targets for antiviral drugs against the SARS-CoV-2 can be RNA polymerase, 3CL Protease and transcription factor Nrf2. Back in early 2020, Chinese, Korean and Ukrainian scientists showed in their works that flavonoids inhibit 3CL protease activity and bind ACE-2, an angiotensin-converting enzyme-2, which acts as a receptor for the coronavirus surface spike glycoprotein, thus promoting the entry of the virus into human cells [18,19].

In preclinical studies, in early 2020, it was found by molecular docking [19] that the active ingredient Proteflazid is well positioned in the catalytic pocket of the 3CL protease of SARS-Cov-2 coronavirus, forming hydrogen bonds with Ser 144, Gly 143 residues, being located between catalytic residues His 163, Asn 142 and Cys 145 and, thus hydrogen bonds with two hydroxyl catechol residues Glu 166, which evidences the inhibition of 3CL protease activity of SARS-Cov-2 coronavirus [19], and during testing the drug based on the active substance Proteflazid, it was established 15 years ago that this substance was an inhibitor of RNA and DNA polymerase of influenza and ARVI viruses.

Materials of research and experimental work of specialists of the SI “L.V. Hromashevskyi IEID of NAMS of Ukraine” showed that the active substance Proteflazid has a high antiviral activity in vitro on a model using transmissible gastroenteritis of swine coronavirus, inhibiting the reproduction of RNA-containing coronavirus (effectiveness was confirmed on two cell cultures) structurally similar to human coronavirus [19].

The Animal Health Research Centre (IRTA-CreSA, Autonomous University of Barcelona, Spain) investigated the specific antiviral activity of Proteflazid against SARS-CoV-2 coronavirus in vitro on Vero E6 cell culture. The drug Proteflazid, drops in a series of successive dilutions in the range 0.0000819 µg – 160 µg was injected into a Vero E6 cell culture with

simultaneous infection with SARS-CoV-2 at an infecting tissue dose of TCID₅₀ /ml 101.8 followed by incubation. Viral infection was assessed microscopically based on the cellular cytotoxic effect. Proteflazid showed antiviral activity against SARS-CoV-2 virus in the concentration range of 1.28 µg – 6.4 µg [20].

US scientists at Galveston National Laboratory focusing on SARS/MERS/COVID-19 (P-3) viruses from the Department of Microbiology and Immunology, Center for Biodefense and Emerging Diseases (Galveston National Laboratory, University of Texas Medical School, Galveston, USA) conducted studies in vitro of African green monkey kidney cell cultures (Vero E6) and human pulmonary adenocarcinoma cells with ACE2 receptor (A549/ACE2) demonstrating the ability of Proteflazid to prevent the virus-induced cytopathic effect caused by SARS-CoV-2 (USA-WA-1/2020 isolate), and provide 100% antiviral protection against cell damage by SARS-CoV-2 virus [21].

Published scientific studies confirm that flavonoids activate the transcription factor Nrf2, thereby reducing the expression of ACE receptors (ACE-2, angiotensin-converting enzyme 2) in respiratory epithelial cells, thus preventing virus penetration into epithelial cells and blocking SARS-CoV-2 virus multiplication, protecting cells from oxidative stress and inflammation, thus reducing the probability of acute respiratory distress syndrome [22-30].

Proteflazid, drops is a direct-acting antiviral agent with the following mechanism of action in acute respiratory viral disease COVID – 19:

a) **blocking the proteolytic activity of 3C-like protease**, which is vital for SARS-CoV-2 virus proliferation [19,24,31-33];

b) **blocking the RNA polymerase** which is necessary for SARS-CoV-2 virus replication [31,34-35];

c) **activation of the transcription factor Nrf2**, which reduces the expression of ACE (angiotensin-converting enzyme 2) receptors in respiratory epithelial cells, thus preventing virus entry into lung epithelial cells and blocking SARS-CoV-2 multiplication, protecting cells from oxidative stress and inflammation, thus reducing the chance of acute respiratory distress syndrome [22-44].

Purpose of the work

Provide a statistical assessment of the effectiveness of the drug Proteflazid®, drops in terms of COVID-19 pandemic.

Stages of work completion:

1. Forming a database for statistical analysis of the results of the use of humanitarian aid in the form of the drug Proteflazid®, drops in terms of the COVID-19 pandemic. The database contains collected, sorted, and generated data presented in the form of tables.

2. Statistical analysis of the results of the use of humanitarian aid in the form of the drug Proteflazid®, drops in terms of the COVID-19 pandemic.

Materials and methods

The database was formed on the basis of 90 letters-references on the effectiveness of the drug Proteflazid®, drops provided by the customer sent by medical institutions from different regions of Ukraine.

Statistical analysis methods were used during the analysis, such as dynamics and structure analysis, meta-analysis, generalization, etc. [45].

Results and discussion

Forming a database for statistical analysis of the results of the use of humanitarian aid in the form of the drug Proteflazid®, drops in terms of the COVID-19 pandemic.

Forming a database for statistical analysis of the results of the use of humanitarian aid in the form of the drug Proteflazid®, drops in terms of the COVID-19 pandemic was carried out on the basis of an analysis of letters sent by medical institutions regarding the use of Proteflazid® for preventive purposes, and directly for therapeutic purposes.

90 letters-references about the effectiveness of the drug Proteflazid®, drops (№ UA/4220/01/01 as of 21.09.2020) were received from medical institutions and analyzed during the prevention and treatment of acute respiratory viral infections, including COVID-19 disease. Some medical institutions sent several letters in different periods. The study used the letters that contained the most complete information. The number of deleted letters-references is 11.

Letters-references from 79 medical institutions from different cities and regions of Ukraine were analyzed, including Vinnytsia Region – 3; Volyn Region – 5; Dnipropetrovsk Region – 5; Donetsk Region – 2; Zakarpattia Region – 1; Zaporizhzhia Region – 2; Ivano-Frankivsk Region – 2; Kirovohrad Region – 1; Luhansk Region – 5; Lviv Region – 1; Odesa Region – 3; Poltava Region – 2; Rivne Region – 7; Ternopil Region – 1; Kharkiv Region – 13; Kherson Region – 2; Khmelnytskyi Region – 2; Cherkasy Region – 1; Chernivtsi Region – 9; Chernihiv Region – 1 and the city of Kyiv – 11 letters.

The period of the beginning of administering the drug by employees and patients of medical institutions was February 27, 2020 (since the first batch of humanitarian aid, in the form of a direct antiviral drug Proteflazid, drops, was transferred to medical workers in Novi Sanzhary [Poltava Region, Ukraine] directly, after the return of Ukrainian citizens and citizens of other countries from Wuhan city, China, for staying under observation due to the possible occurrence of COVID – 19).

Final information – October 01, 2020.

The vast majority of letters from medical institutions indicated that medical staff were in contact with patients potentially suffering from COVID-19. This means a high risk of being infected with Coronavirus infection.

I. The following can be noted on the analysis results of the information base regarding medical professionals and patients who were administered the drug Proteflazid® for preventive purposes.

Total number of patients who took Proteflazid® for preventive purpose was 8,572, including 7,444 medical workers and 1,128 ordinary patients.

Indicator "Number of fatalities" for the medical institutions providing such information was "0".

II. The following can be noted on the analysis results of the information base regarding medical professionals and patients who were administered the drug Proteflazid® for therapeutic purposes.

Total number of patients who took Proteflazid® for therapeutic purposes was 433, including 23 medical workers and 410 ordinary patients.

Indicator "Number of fatalities" for the medical institutions providing such information was "0".

The total number of medical personnel and patients who used Proteflazid drops for preventive and therapeutic purposes was 9005 people.

Statistical analysis of the results of the use of humanitarian aid in the form of the drug Proteflazid®, drops in terms of the COVID-19 pandemic.

For the analysis, actual data as of 01.10.2020 were used, since just in this period the information generated in information databases regarding medical professionals and patients who took Proteflazid® for preventive purposes, and directly for therapeutic purposes.

Table 1 presents the summary of the main indicators of COVID-19 global incidence as of 01.10.2020, ranked in descending order by such an indicator as the number of patients.

Table 1. The main indicators of COVID-19 global incidence as of 01.10.2020 incidence as of 01.10.2020

Country	Number of patients	Number of recovered	Number of fatalities	Percentage of recovered	Proportion of fatalities
The USA	7 484 357	4 725 190	212 393	63,1	2,8
India	6 391 960	5 348 653	99 804	83,7	1,6
Brazil	4 820 116	4 180 376	144 103	86,7	3,0
Russia	1 185 231	964 242	20 891	81,4	1,8
Colombia	829 679	743 653	25 998	89,6	3,1
Peru	814 829	683 815	32 463	83,9	4,0
Spain	778 607	–	31 973	–	4,1
Argentina	751 001	594 645	16 937	79,2	2,3
Mexico	743 216	533 886	77 646	71,8	10,4
RSA	676 084	609 584	16 866	90,2	2,5
France	577 505	96 797	32 019	16,8	5,5
Chile	464 750	438 148	12 822	94,3	2,8
Iran	461 044	383 368	26 380	83,2	5,7
United Kingdom	460 178	–	42 202	–	9,2
Iraq	367 474	295 882	9 231	80,5	2,5
Bangladesh	364 987	277 078	5 272	75,9	1,4
Saudi Arabia	335 097	319 746	4 794	95,4	1,4
Turkey	320 070	281 151	8 262	87,8	2,6
Italy	317 409	228 844	35 918	72,1	11,3
Philippines	314 079	254 223	5 562	80,9	1,8

Pakistan	312 806	297 497	6 484	95,1	2,1
Germany	295 530	257 900	9 586	87,3	3,2
Indonesia	291 182	218 487	10 856	75,0	3,7
Israel	253 490	179 468	1 622	70,8	0,6
Ukraine*	217661	96 591	4 261	44,4	2,0
Ukraine**	213 028	94 443	4 193	44,3	2,0
Global	34 400 568	25 589 531	1 022 512	74,4	3,0
*, ** <i>Source [34]</i>					

As of 1.10.2020 Ukraine was among the top 25 in terms of the number of patients. The largest number of cases was observed in the United States. In Ukraine, the proportion of recovered patients has been too small at the level of 44.3% when compared to 74.4% in the whole world. And in some countries (Saudi Arabia, Pakistan and Chile), it even reaches 95%.

The proportion of fatalities in Ukraine is 2% (global value is 3%). The highest number of fatalities was observed in Italy (11.3%) and Mexico (10.4%), and the lowest – in Israel (0.6%).

Table 2 presents the main indicators of COVID-19 incidence in Ukraine as of 01.10.2020.

Table 2. The main indicators of COVID-19 incidence in Ukraine as of 01.10.2020

Region	Number of patients	Number of recovered	Number of fatalities	Percentage of recovered	Proportion of fatalities
Vinnysia Region	6 308	4041	116	64,1	1,8
Volyn Region	8 194	5 253	170	64,1	2,1
Dnipropetrovsk Region	6 088	3 221	114	52,9	1,9
Donetsk Region	3 557	1 117	56	31,4	1,6
Zhytomyr Region	6 088	3 433	111	56,4	1,8
Zakarpattia Region	9 918	4 798	308	48,4	3,1
Zaporizhzhia Region	4 128	1 429	64	34,6	1,6
Ivano-Frankivsk Region	13 837	6 536	318	47,2	2,3
Kyiv Region	9 279	5 639	178	60,8	1,9
Kirovohrad Region	1 127	877	57	77,8	5,1
Luhansk Region	1 091	576	14	52,8	1,3
Lviv Region	20 590	7 387	565	35,9	2,7
Mykolaiv Region	3 270	1 086	77	33,2	2,4
Odesa Region	13 789	2 550	208	18,5	1,5

Poltava Region	2 345	1 072	42	45,7	1,8
Rivne Region	12 554	10 064	167	80,2	1,3
Sumy Region	4 435	1 798	75	40,5	1,7
Ternopil Region	13 767	8 183	170	59,4	1,2
Kharkiv Region	19 128	4 363	354	22,8	1,9
Kherson Region	1 168	616	26	52,7	2,2
Khmelnyskyi Region	6 188	2 645	120	42,7	1,9
Cherkasy Region	4 165	1 740	57	41,8	1,4
Chernivtsi Region	14 605	8 115	364	55,6	2,5
Chernihiv Region	4 254	963	71	22,6	1,7
Kyiv	23 155	6 941	391	30,0	1,7
Ukraine*	217 661	96 591	4 261	44,4	2,0
Ukraine**	213 028	94 443	4 193	44,3	2,0
*, ** <i>Source [34]</i>					

As of 1.10.2020, the largest number of patients falls on the city of Kyiv, and Lviv and Kharkiv Regions. The largest proportion of recovered falls to Rivne Region (80.2%), and the smallest one – to Odesa Region (18.5%). The largest proportion of fatalities is observed in Kirovohrad Region (5.1%), and the smallest one – in Ternopil Region (1.2%).

Table 3 presents a comparison of the proportion of recovered patients among those who took Proteflazid® for prevention, and the proportion of recovered patients on a country-by-country basis.

Table 3. Comparison of the proportion of recovered patients among those who took Proteflazid® for prevention, and the proportion of recovered patients on a country-by-country basis as of 01.10.2020

Country	Average proportion of the recovered patients among those who took Proteflazid® for preventive purposes (Ukraine), %	Proportion of patients recovered on a country-by-country basis, %	Difference (Ukraine/ country from the list), fold
The USA	86,1	63,1	1,36
India	86,1	83,7	1,03
Brazil	86,1	86,7	0,99
Russia	86,1	81,4	1,06
Colombia	86,1	89,6	0,96
Peru	86,1	83,9	1,03
Spain	86,1	—	—
Argentina	86,1	79,2	1,09
Mexico	86,1	71,8	1,20
RSA	86,1	90,2	0,95
France	86,1	16,8	5,13

Chile	86,1	94,3	0,91
Iran	86,1	83,2	1,03
United Kingdom	86,1	–	–
Iraq	86,1	80,5	1,07
Bangladesh	86,1	75,9	1,13
Saudi Arabia	86,1	95,4	0,90
Turkey	86,1	87,8	0,98
Italy	86,1	72,1	1,19
Philippines	86,1	80,9	1,06
Pakistan	86,1	95,1	0,91
Germany	86,1	87,3	0,99
Indonesia	86,1	75,0	1,15
Israel	86,1	70,8	1,22
Ukraine	86,1	44,4	1,94
Global	86,1	74,4	1,16
<i>Source:</i> Information database of medical professionals and patients who took Proteflazid® for preventive purposes [6, 26, 34].			

The proportion of recovered among those who took Proteflazid® for the prevention of COVID-19 is 86.1%. It is slightly underestimated, since not all medical institutions provided information on the number of recovered patients. The proportion of those who recovered among those who took Proteflazid® for the prevention of COVID-19 is 1.94 times higher than the proportion of those who recovered in Ukraine as a whole and 1.16 times higher than in the whole world. At the same time, by this indicator, it almost reaches the value of the most effective countries.

Table 4 presents a comparison of the proportion of recovered patients among those who took Proteflazid® directly for therapeutic purposes, and the proportion of recovered patients on a country-by-country basis.

Table 4. Comparison of the proportion of recovered patients among those who took Proteflazid® directly for therapeutic purposes, and the proportion of recovered patients on a country-by-country basis as of 01.10.2020

Country	Average proportion of the recovered patients among those who took Proteflazid® directly for therapeutic purposes (Ukraine), %	Proportion of patients recovered on a country-by-country basis, %	Difference (Ukraine/country from the list), fold
The USA	90,8	63,1	1,44
India	90,8	83,7	1,08
Brazil	90,8	86,7	1,05
Russia	90,8	81,4	1,12
Colombia	90,8	89,6	1,01
Peru	90,8	83,9	1,08
Spain	90,8	–	–

Argentina	90,8	79,2	1,15
Mexico	90,8	71,8	1,26
RSA	90,8	90,2	1,01
France	90,8	16,8	5,40
Chile	90,8	94,3	0,96
Iran	90,8	83,2	1,09
United Kingdom	90,8	—	—
Iraq	90,8	80,5	1,13
Bangladesh	90,8	75,9	1,20
Saudi Arabia	90,8	95,4	0,95
Turkey	90,8	87,8	1,03
Italy	90,8	72,1	1,26
Philippines	90,8	80,9	1,12
Pakistan	90,8	95,1	0,95
Germany	90,8	87,3	1,04
Indonesia	90,8	75,0	1,21
Israel	90,8	70,8	1,28
Ukraine	90,8	44,4	2,05
Global	90,8	74,4	1,22

Source:

Information database of medical professionals and patients who took Proteflazid® directly for therapeutic purposes [6, 26, 34].

The proportion of recovered among those who took Proteflazid® directly for therapeutic purposes against COVID-19 is 90.8%. It is slightly underestimated, since not all medical institutions provided information on the number of recovered patients. The proportion of those who took Proteflazid® directly for therapeutic purposes against COVID-19 is 2.05 times higher than the proportion of those who recovered in Ukraine as a whole and 1.22 times higher than in the whole world.

Table 5 presents a comparison of the proportion of those who took Proteflazid® for preventive purposes, and the proportion of recovered patients on a country-by-country basis.

Table 5. Comparison of the proportion of those who took Proteflazid® for preventive purposes, and the proportion of recovered patients on a country-by-country basis as of 01.10.2020

Region	Average proportion of the recovered patients among those who took Proteflazid® for preventive purposes, %	Proportion of patients recovered on a region-by-region basis, %	Difference, fold
Vinnysia Region	86,1	64,1	1,34
Volyn Region	86,1	64,1	1,34
Dnipropetrovsk Region	86,1	52,9	1,63
Donetsk Region	86,1	31,4	2,74
Zhytomyr Region	86,1	56,4	1,53

Zakarpattia Region	86,1	48,4	1,78
Zaporizhzhia Region	86,1	34,6	2,49
Ivano-Frankivsk Region	86,1	47,2	1,82
Kyiv Region	86,1	60,8	1,42
Kirovohrad Region	86,1	77,8	1,11
Luhansk Region	86,1	52,8	1,63
Lviv Region	86,1	35,9	2,40
Mykolaiv Region	86,1	33,2	2,59
Odesa Region	86,1	18,5	4,65
Poltava Region	86,1	45,7	1,88
Rivne Region	86,1	80,2	1,07
Sumy Region	86,1	40,5	2,13
Ternopil Region	86,1	59,4	1,45
Kharkiv Region	86,1	22,8	3,78
Kherson Region	86,1	52,7	1,63
Khmelnyskyi Region	86,1	42,7	2,02
Cherkasy Region	86,1	41,8	2,06
Chernivtsi Region	86,1	55,6	1,55
Chernihiv Region	86,1	22,6	3,81
Kyiv	86,1	30,0	2,87
Ukraine	86,1	44,4	1,94

Source:

Information database of medical professionals and patients who took Proteflazid® for preventive purposes [6, 26, 34].

The proportion of recovered among those who took Proteflazid® for preventive purposes against COVID-19 is 1.07 times higher than the highest proportion of those who recovered in Ukraine (Rivne Region) and 4.65 times higher than the lowest proportion of those who recovered in Ukraine (Odesa Region).

Table 6 presents a comparison of the proportion of those who took Proteflazid® directly for therapeutic purposes, and the proportion of recovered patients in the regions of Ukraine.

Table 6. Comparison of the proportion of those who took Proteflazid® directly for therapeutic purposes, and the proportion of recovered patients in the regions of Ukraine as of 01.10.2020

Region	Average proportion of the recovered patients among those who took Proteflazid® directly for therapeutic purposes (Ukraine) , %	Proportion of patients recovered on a region-by-region basis, %	Difference, fold
Vynnytsia Region	90,8	64,1	1,42
Volyn Region	90,8	64,1	1,42
Dnipropetrovsk Region	90,8	52,9	1,72

Donetsk Region	90,8	31,4	2,89
Zhytomyr Region	90,8	56,4	1,61
Zakarpattia Region	90,8	48,4	1,88
Zaporizhzhia Region	90,8	34,6	2,62
Ivano-Frankivsk Region	90,8	47,2	1,92
Kyiv Region	90,8	60,8	1,49
Kirovohrad Region	90,8	77,8	1,17
Luhansk Region	90,8	52,8	1,72
Lviv Region	90,8	35,9	2,53
Mykolaiv Region	90,8	33,2	2,73
Odesa Region	90,8	18,5	4,91
Poltava Region	90,8	45,7	1,99
Rivne Region	90,8	80,2	1,13
Sumy Region	90,8	40,5	2,24
Ternopil Region	90,8	59,4	1,53
Kharkiv Region	90,8	22,8	3,98
Kherson Region	90,8	52,7	1,72
Khmelnitskyi Region	90,8	42,7	2,13
Cherkasy Region	90,8	41,8	2,17
Chernivtsi Region	90,8	55,6	1,63
Chernihiv Region	90,8	22,6	4,02
Kyiv	90,8	30,0	3,03
Ukraine	90,8	44,4	2,05

Source:

Information database of medical professionals and patients who took Proteflazid® directly for therapeutic purposes [6, 26, 34].

The proportion of recovered among those who took Proteflazid® directly for therapeutic purposes against COVID-19 is 1.13 times higher than the highest proportion of those who recovered in Ukraine (Rivne Region) and 4.91 times higher than the lowest proportion of those who recovered in Ukraine (Odesa Region).

All these results confirm the effectiveness of using the drug Proteflazid® for the prevention and treatment of COVID-19.

The effectiveness of the drug Proteflazid® for the treatment of COVID-19 is evidenced by the absence of fatalities (information bases of medical workers and patients who took "Proteflazid®" for preventive purposes and directly for therapeutic purposes) when compared to 2% in Ukraine and 3% in the world as a whole (Table. 2.1).

To assess the effectiveness of the drug Proteflazid® in terms of the COVID-19 pandemic, a meta-analysis was used, which in modern studies is used to generalize the effectiveness of the results of various groups of objects. In our case, this method is used for a generalized assessment of the effectiveness of the drug "Proteflazid®" in the regions of Ukraine.

As the main indicator for assessing the effectiveness of the drug Proteflazid®, RR (Risk Ratio) was chosen – the risk ratio which demonstrates how many times the proportion of those who recovered differs among those who took "Proteflazid®" for preventive purposes and directly for therapeutic purposes, compared with the general population of patients with COVID-19 in Ukraine on a region-by-region basis. This indicator is calculated separately for each region using the formula:

$$RR = \frac{a \times N}{b \times M} = \frac{a \times 100}{b \times 100} = \frac{a}{b}$$

where:

a – the proportion of patients who recovered among those who took Proteflazid® for preventive purposes and directly for therapeutic purposes in the corresponding region, %;

b – the proportion of those who recovered among the total population of COVID-19 cases in Ukraine in the corresponding region, %;

N – the maximum proportion of recovered patients among those who took Proteflazid® for preventive purposes and directly for therapeutic purposes in the corresponding region ($N = 100$), %;

M – the maximum proportion of those who recovered from the total population of COVID-19 cases in Ukraine in the corresponding region ($M = 100$), %.

The total RR value for regions is calculated taking into account the weight coefficients for each region (Weight).

If the risk ratio (RR) is 1, then there are no differences. The closer to zero and the further away from one, the stronger the differences.

If the RR exceeds 1, then there is a predominance of the proportion of recovered patients among those who took Proteflazid® for preventive purposes and directly for therapeutic purposes, when compared with the general population of patients with COVID-19 in Ukraine on a region-by-region basis. The difference values in this case will be equal to the RR value.

If the RR is 0 to 1, then there is a decrease in the proportion of recovered patients among those who took Proteflazid® for preventive purposes and directly for therapeutic purposes, when compared with the general population of patients with COVID-19 in Ukraine on a region-by-region basis. The value of the difference, in this case, will be equal to the ratio of 1 to RR.

Calculations were performed with the use of the RevMan software.

The main results of meta-analysis, including the value of the risk ratio (RR) and the generalized risk value ("big rhombus") are shown in the Forest plot graphs (fig. 1 and 2).

The vertical line in the middle of the graph ($RR = 1$) corresponds to the situation when the proportions of those who recovered among those who took Proteflazid® for preventive or directly for therapeutic purposes do not differ from the proportions of the total population of COVID-19 patients in Ukraine on a region-by-region basis. The greater the statistical weight for this region in the general pool, the larger the size of the mark for each specific region. Horizontal lines ("whiskers") near the mark of each region show the 95% confidence interval. The "big rhombus" mark is a generalized total, with the length of the rhombus reflecting the combined estimate of the 95% confidence interval. The lower horizontal scale assesses the difference between the two populations. The area to the left of the vertical "1" corresponds to a decrease in the recovery rate among those who took Proteflazid® for preventive purposes or directly for therapeutic purposes, compared with the total number of COVID

– 19 cases in Ukraine on a region-by-region basis. The area to the right of the vertical "1" corresponds to an increase in the recovery rate among those who took "Proteflazid®" for preventive purposes or directly for therapeutic purposes, compared with the total number of COVID – 19 cases in Ukraine on a region-by-region basis.

In the lower-left corner in Fig. 1 and 2 generalized values of statistical criteria for checking the uniformity of the recovery rates in the regions of Ukraine for two populations (χ^2 criterion and I^2), and the statistical significance of differences in populations (Z-criterion) are given. The main indicators of these criteria are the values P and I^2 . If P -value of χ^2 and Z -criteria is less than 0.05, this indicates a statistically significant heterogeneity of data and significant differences between the recovery rate among those who took Proteflazid® for prevention or treatment purposes, compared with the total number of COVID – 19 cases in Ukraine by regions with COVID-19 in Ukraine on an region-by-region basis. If the result of the I^2 test exceeds 50%, this indicates the existence of heterogeneity among the data. In this case, a model with a random effect is selected. Otherwise, a model with a fixed effect is selected.

To assess the effectiveness of treatment with the drug Proteflazid® of COVID-19 patients among those who took the drug for prevention purposes, information from 7 regions of Ukraine (Vinnytsia, Dnipropetrovsk, Rivne, Ternopil, Khmelnytskyi, Cherkasy, Chernivtsi) and the city of Kyiv (fig. 1). In 13 regions of Ukraine (Volyn, Donetsk, Zakarpattia, Zaporizhzhia, Ivano-Frankivsk, Kirovohrad, Luhansk, Lviv, Odesa, Poltava, Kharkiv, Kherson and Chernihiv) as a result of taking the drug Proteflazid® for prevention purposes, not a single person fell ill.

To assess the effectiveness of treatment with the drug Proteflazid® of COVID-19 patients among those who took the drug directly for therapeutic purposes, information from 6 regions of Ukraine (Vinnytsia, Zaporizhzhia, Odesa, Poltava, Chernivtsi, Chernihiv) and the city of Kyiv (fig. 2).

Recovery rates among those who took Proteflazid® for the prevention and treatment of COVID-19 are underestimated in some areas, since not all medical institutions provided information.

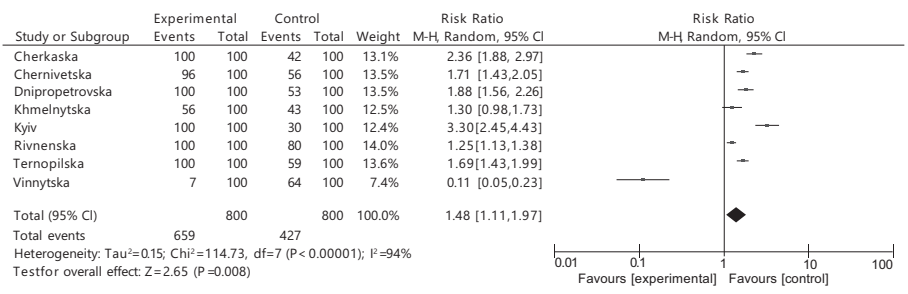


Figure. 1. Results of a meta-analysis of comparison of recovery rate among those who took Proteflazid® for the preventive purposes, and in total amount of COVID-19 cases in Ukraine on a region-by-region basis

Source:
Information database of medical professionals and patients who took Proteflazid® for preventive purposes [6, 34].

χ – squared value of the criterion ($P=0.000$) and I^2 -test ($I^2= 94\%$) indicate that the study data is heterogeneous, so a model with a random effect was chosen. P – value of the Fisher criterion ($P= 0.008$) demonstrates the significance of the selected effect.

Value of the risk ratio (RR = 1.48) indicates that the recovery rate among those who took Proteflazid® directly for the prevention purposes is 1.48 times higher than the proportion in the total amount of COVID-19 cases in Ukraine.

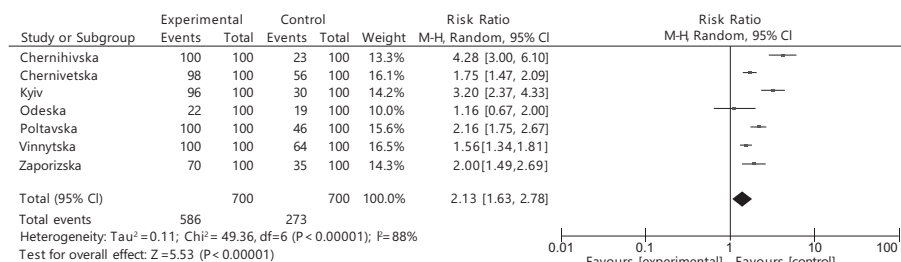


Figure 2. Results of a meta-analysis of comparison of recovery rate among those who took Proteflazid® for the therapeutic purposes, and in total amount of COVID-19 cases in Ukraine on a region-by-region basis

Source:

Information database of medical professionals and patients who took Proteflazid® directly for therapeutic purposes [6, 34].

χ – squared value of the criterion ($P = 0.000$) and I^2 -test ($I^2 = 88\%$) indicate that the study data is heterogeneous, so a model with a random effect was chosen. P – value of the Fisher criterion ($P = 0.0000$) demonstrates the significance of the selected effect.

Value of the risk ratio (RR = 2.13) indicates that the recovery rate among those who took Proteflazid® directly for the prevention purposes is 2.13 times higher than the proportion in the total amount of COVID-19 cases in Ukraine.

So, the statistical analysis shows in favour of the use of the drug Proteflazid®, since the recovery rate increases as a result of the drug use in comparison with the recovery rate in the total population of patients with COVID-19 in Ukraine.

Figure 3 shows a comparison of the proportion of ill medical workers who took Proteflazid® for preventive purposes, with the proportion of medical workers infected with COVID-19 in the total population of medical workers in Ukraine.

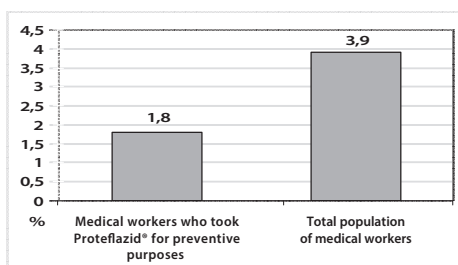


Figure 3. Comparison of the proportion of ill medical workers who took Proteflazid® for preventive purposes, with the proportion of medical workers infected with COVID-19 in the total population of medical workers in Ukraine

Source:

Information database of medical professionals and patients who took Proteflazid® for preventive purposes [30, 43].

The proportion of ill medical workers who took Proteflazid® for preventive purposes was 2.2 times less than the proportion of medical workers infected with COVID-19 in the total population of medical workers in Ukraine. Since equal-probability events were selected as comparison items, the assessment confirms the effectiveness of the use of the drug Proteflazid® for prevention purposes.

Fig. 4 shows the comparison of the proportion of doctors with severe disease, and patients who took Proteflazid® for the prevention purposes, with the proportion of patients with severe disease in the total population of patients with COVID-19.

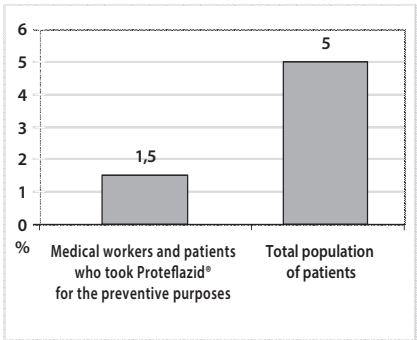


Figure 4. The comparison of the proportion of medical workers with severe disease, and patients who took Proteflazid® for the preventive purposes, with the proportion of patients with severe disease in the total population of patients with COVID-19.

Source:
Information database of medical professionals and patients who took Proteflazid® for preventive purposes [30].

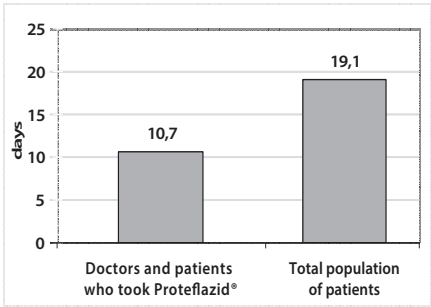


Figure 5. Average duration of treatment of doctors and patients who took Proteflazid® was compared with the average duration of treatment in the total population of COVID-19 patients.

Source:
Information database of medical professionals and patients who took Proteflazid® for preventive purposes [6, 34]

The proportion of medical workers with severe disease, and patients who took Proteflazid® for the preventive purposes is 3.3 times lower than the proportion of patients with severe disease in the total population of patients. Performed calculations also confirm the effectiveness of the use of the drug Proteflazid® for the preventive purposes.

Fig. 5 shows the average duration of treatment of doctors and patients who took Proteflazid® was compared with the average duration of treatment in the total population of COVID-19 patients. The average duration of COVID-19 cases in the total population of cases was calculated using the weighted average formula.

The average duration of treatment of medical workers and patients who took Proteflazid® is 1.8 times lower than the average duration of treatment for COVID-19 patients in the total population of patients. Performed calculations also confirm the effectiveness of the use of the drug Proteflazid® for both preventive and therapeutic purposes.

Conclusions

It was found out that as of 01.10.2020 Ukraine was among the top 25 in terms of the number of patients. At the same time, the proportion of recovered patients has been too small at the level of 44.3% when compared to 74.4% in the whole world. And in some countries, it even reaches 95%. During this period, the share of fatalities was 2%. All this requires immediate measures to improve the effectiveness of treatment of COVID-19 patients in Ukraine.

During the period declared a pandemic by WHO due to the spread of the SARS-CoV-2 coronavirus that causes COVID-19 disease worldwide, “SMC “Ecopharm” Ltd. provided charitable assistance in the form of 32,995 packages of the drug Proteflazid®, drops (no. UA/4220/01/01 from 21.09.2020), in order to prevent and treat the diseases caused by acute respiratory viral infection (ARVI), including those caused by COVID-19.

90 reference letters were received from medical institutions. 79 letters-references about the effectiveness of the drug Proteflazid®, drops, during the prevention and treatment of acute respiratory viral infections, including COVID-19 diseases were analyzed. The references indicate the effectiveness of the use of the drug Proteflazid®, drops, for prevention purposes among medical personnel in the number of 7444 persons, patients in the number of 1128 persons; treatment of mild, moderate, severe course of the disease in patients during prevention in the number of 137 persons, who fell ill against the background of preventive use of the drug; and direct treatment of patients with a mild, moderate, or severe course of the disease in the number of 433 persons; under zero mortality under preventive and therapeutic use of the drug Proteflazid®, drops.

Performed statistical analysis confirms the effectiveness of the drug Proteflazid® for the prevention and treatment of COVID-19, as, when compared with official actual data, regarding the main indicators of the incidence of COVID-19: there were no fatalities; the average treatment period decreased (1.8 times); the proportion of recovered increased (at least 1.5 times); the proportion of sick medical workers in the total population of sick medical workers decreased (2.2 times); the proportion of patients with a severe course decreased (3.3 times).

According to the aforementioned statistical data, it can be argued that the drug Proteflazid®, drops has shown high effectiveness in the prevention and treatment of acute respiratory viral infections, including COVID-19, among medical personnel and patients.

From the above information, the main conclusion follows: out of 9005 persons who used the drug Proteflazid, drops for the prevention and treatment of acute respiratory viral diseases (including COVID-19), not a single fatal case was registered.

With this in mind, we can give recommendations regarding the "off-label" use of the drug Proteflazid, drops for the prevention and treatment of COVID-19 disease:

preventive reception – 15 drops twice daily (for a month, followed by a weekly break);

preventive treatment – immediately after contact with a COVID-19 patient, when no symptoms of the disease have yet appeared, 30 drops twice daily, for 15 days;

treatment – from the first hours of the onset of the disease symptoms, 30 drops twice daily, for 15 days.

In some cases, the use of the drug may be continued to prevent complications due to possible infection of the patient with other viral pathogens.

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References:

1. Abian O., Ortega-Alarcon D., Jimenez-Alesanco A. et al. Structural stability of SARS-CoV-2 3CLpro and identification of quercetin as an inhibitor by experimental screening. // International Journal of Biological Macromolecules. – 2020. – Vol. 164. – P. 1693–1703.
2. Beketova G., Grynevych O., Solomakha L., Golovnia N. Proteflazid®: Clinical experience in children of young and preschool age – systematic review of postmarketing surveillance // Polski Merkuriusz Lekarski. – 2018. – Tom XLIV. – №260. – P. 75–81.
3. Chen S., Short J. A., Young D. F., Killip M. J., Schneider M., Goodbourn S., Randall R. E. Heterocellular induction of interferon by negative-sense RNA viruses. // Virology. – 2010. – 407, 247–255. [CrossRef]
4. Chien-Te Kent Tseng, Dr. Aleksandra K. Drelich. Report «The efficacy assessment of new compound against SARS-CoV-2 infection in *in vitro* models (PROJECT #74908)» // Galveston National Laboratory, Galveston, USA. – 2021. – P. 1–4.
5. Commitment and Call to Action: Global Collaboration to Accelerate New COVID-19 Health Technologies; <https://www.who.int/news-room/detail/24-04-2020-commitment-and-call-to-action-global-collaboration-to-accelerate-new-covid-19-health-technologies> (accessed 16 June 2020).
6. COVID-19 coronavirus pandemic. URL: <https://www.worldometers.info/coronavirus>.
7. Grynevych O., Kramarev S., Matyash V. [et al.] PROTEFLAZID®: Specific activity in Epstein-Barr virus infection in a preclinical study; efficacy and safety in the clinic (systematic review) // Japanese Educational and Scientific Review. – 2015. – V. No. 1 (9). – P. 113–126.
8. Jia D., Rahbar R., Chan R. W., Lee S. M., Chan M. C., Wang B. X., Baker D. P., Sun B., Peiris J. M., Nicholls J. M. Influenza virus non-structural protein 1 (NS1) disrupts interferon signaling. // PLoS ONE. – 2010. – 5, e13927. [CrossRef]
9. Kaminsky V. V., Litus O. I., Grynevych O. Y. [et al.] PROTEFLAZID®: efficacy and safety in urogenital herpes virus infection (systematic review) // Science and Education Studies. – 2015. – V3, № 2. (16). – P. 705–727.
10. Karamuska T. Interim report «Evaluation of the antiviral activity of a test item against SARS-CoV-2 *in vitro* in Vero E6 cells» // IRTA-CreSA Universitat Autònoma de Barcelona, Barcelona, Spain. – 2020. – P. 8.

11. Kramarev S., Mikhailov V., Grynevych O. [et al.] Meta-Analysis of clinical trials results of efficacy and safety of the drug Proteflazid® in the treatment and prevention of human herpesvirus infection in children // *Asian Journal of Scientific and Educational Research*. – 2016. – №1(19), January-June. – P.766–783.
12. Lee C. Therapeutic Modulation of Virus-Induced Oxidative Stress via the Nrf2 – Dependent Antioxidative Pathway. // *Oxidative Med. Cell. Longev*. – 2018, 2018, 6208067.
13. Li F. Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits. // *J. Virol*. – 2012. – 86. – 2856–2858. [CrossRef]
14. Li W., Moore M. J., Vasilieva N., Sui J., Wong S. K., Berne M. A., Somasundaran M., Sullivan J. L., Luzuriaga K., Greenough T. C. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. // *Nature*. – 2003. – 426. – 450–454. [CrossRef]
15. Matyash V., Grynevych O., Broun T. Proteflazid®: treatment of herpesvirus and mixed infections. Meta-analysis of clinical trials results // *Polski Merkuriusz Lekarski*. – 2018. – Tom X88LIV. – №263. – P.236–247.
16. Matyash V., Grynevych O., Panasyuk O. [et all]. Proteflazid®: specific activity against Herpes virus in preclinical investigations and its efficacy/safety in clinical practice (systematic review) // *Yale Review of Education and Science*. – 2015. – V.VI, №.1(16). – P. 422–462.
17. McCord J. M., Hybertson B. M., Cota-Gomez A., Gao B. Nrf2 Activator PB125® as a Potential Therapeutic Agent Against COVID-19. // *bioRxiv*. – 2020. [CrossRef]
18. Nguyen T. T., Woo H. J., Kang H. K., Nguyen V. D., Kim Y. M., Kim D. W., Ahn S. A., Xia Y., Kim D. Flavonoid-mediated inhibition of SARS coronavirus 3C-like protease expressed in *Pichia pastoris*. // *Biotechnol. Lett*. – 2012. – 34, 831–838. [CrossRef] [PubMed]
19. Patricia Mendonca and Karam F. A. Soliman. Flavonoids Activation of the Transcription Factor Nrf2 as a Hypothesis Approach for the Prevention and Modulation of SARS-CoV-2 Infection Severity // *Antioxidants*. – 2020. – 9, 659; doi:10.3390/antiox9080659.
20. Ryu Y. B., Jeong H. J., Kim J. H., Kim Y. M., Park J. Y., Kim D., Nguyen T. T., Park S. J., Chang J. S., Park K. H. Biflavonoids from *Torreya nucifera* displaying SARS-CoV 3CL(pro) inhibition. // *Bioorg. Med. Chem*.
21. Silva-Palacios A., Ostolga-Chavarria M., Zazueta C., Konigsberg M. Nrf2: Molecular and epigenetic regulation during aging. // *Ageing Res. Rev*. – 2018. – 47. – P. 31–40.
22. Ventskovsky B., Grynevych O., Mikhailov V. [et al.] Proteflazid® in the treatment and prevention of human herpesvirus infection in pregnant women: a meta-analysis of clinical trials results // *American Journal of Science and Technologies*. – 2016. – №1 (21), January-June. – C. 983–1002.
23. Wang M. M., Lu M., Zhang C. L., Wu X., Chen J. X., Lv W. W., Sun T., Qiu H., Huang S. H. Oxidative stress modulates the expression of toll-like receptor 3 during respiratory syncytial virus infection in human lung epithelial A549 cells. // *Mol. Med. Rep*. – 2018. – 18. – 1867–1877. [CrossRef] [PubMed]
24. Wrapp D., Wang N., Corbett K. S., Goldsmith J. A., Hsieh C.-L., Abiona O., Graham B. S., McLellan J. S. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. // *Science*. – 2020. – 367. – 1260–1263. [CrossRef] [PubMed]
25. Zakaryan H., Arabyan E., Oo A., Zandi K. Flavonoids: Promising natural compounds against viral infections. // *Arch. Virol*. – 2017. – 162, 2539–2551. [CrossRef]
26. Аналітичні панелі та відкриті дані. URL: <https://www.covid19.gov.ua/analitichni-paneli-dashbordy>.
27. Бекетова Г. В., Хайтович М. В., Гриневич О. Й. Імунофлазід в педіатрії: системний аналіз ефективності та безпеки застосування. // *Східна Європа*. – 2014. – № 3 (07). – С. 141–152.

28. Бенюк В. О., Кувіта Ю. В., Гриневич О. Й. [та ін.] Протефлазид®: мета-аналіз результатів клінічних досліджень для оцінки ефективності та безпеки застосування у вагітних жінок. – 2014. – №7 (93). – С. 166–174.
29. Дерябін О. М., Завелевич М. П., Старосила Д. Б. та ін. Природні поліфеноли як інгібітори взаємодії коронавірусів з клітинами: огляд літератури та експериментальні дані. // Укр.мед. часопис. – 2020. – №3 (137), Т.1. – С. 1–5.
30. Здоров'я. URL: https://healts.24tv.ru/v_ukraine_788_medikov_zaboleli_koronavirusom_n1327331
31. Камінський В. В., Шалько М. М., Воробйова Л. І. [та ін.] ПРОТЕФЛАЗИД®: специфічна активність у доклінічних дослідженнях, ефективність та безпека застосування в клінічній практиці при захворюваннях, спричинених вірусом папіломи людини (систематичний огляд) // Здоров'я жінки. – 2015. – №3 (99). – С. 122–132.
32. Камінський В. В., Шалько М. М., Михайлов В. С. Оцінка ефективності препарату ПРОТЕФЛАЗИД® при лікуванні папіломавірусної інфекції: метааналіз результатів багаторічних клінічних досліджень // Медичні аспекти здоров'я жінки. – 2015. – №6 (92). – С. 5–14.
33. Камінський В. В., Шалько М. Н., Гриневич О. Й. ПРОТЕФЛАЗИД® у вагітних: системний огляд результатів післяреєстраційних спостережень за безпечністю та ефективністю застосування // Здоров'я жінки. – 2014. – №6 (92). – С. 160–164.
34. Коронавірус COVID-19: загальна статистика.
URL: <https://index.minfin.com.ua/reference/coronavirus>
35. Крамарьов С. О., Гриневич О. Й., Тонковід О. Б. [та ін.] Метааналіз результатів клінічних досліджень ефективності флавоноїдів при вірусних та вірусно-бактеріальних захворюваннях у дітей. – 2014. – №5(61). – С. 1–7.
36. McCord J. M., Hybertson B. M., Cota-Gomez A., Gao B. Nrf2 Activator PB125® as a Potential Therapeutic Agent Against COVID-19. // bioRxiv. – 2020. [CrossRef]
37. Момотюк Л. Є. Метааналіз результатів клінічних досліджень впливу флавоноїдів на показники клітинної ланки імунітету у дорослих, вагітних жінок та дітей при вірусних та вірусно-бактеріальних захворюваннях // Звіт. Національна академія статистики, обліку та аудиту. – Київ. – 2017. – С. 71.
38. Михайлов В. С. Метааналіз результатів клінічних досліджень ефективності та безпеки застосування лікарського препарату ПРОТЕФЛАЗИД® при лікуванні вірусних гепатитів // Звіт. ДП «НДП статистичних досліджень». – Київ. – 2015. – С. 104.
39. Момотюк Л. Є., Ященко Л. О., Мотузка О. М. Заклучний звіт «Формування бази даних та статистичний аналіз ефективності використання препарату ПРОТЕФЛАЗИД®, краплі в умовах COVID-19». // Національна академія статистики, обліку та аудиту. Київ. – 2020. – С. 38.
40. Пальчиковська Л. Г., Васильченко О. В., Платонов М. О. та ін. Антивірусні властивості рослинних флавоноїдів – інгібіторів синтезу ДНК та РНК // Biopolymers and Cell. – 2013. – V.29, (2). – Р. 150–156.
41. Печінка А. М., Гриневич О. Й., Крючко Т. А. [та ін.] ПРОТЕФЛАЗИД®: специфічна активність щодо вірусу гепатиту С у доклінічних дослідженнях; ефективність та безпека при лікуванні гепатитів В та С у клінічній практиці (систематичний огляд) // Клінічна інфектологія та паразитологія. – 2015. – № 2 (13). – С. 80–99.
42. Рибалко С. Л. «Звіт про вивчення механізмів дії біологічно активних речовин лікувальної субстанції протефлазиду. // Інститут епідеміології та інфекційних хвороб ім. Л. В. Громашевського НАМН України. – м. Київ. – 2010. – С. 83.

43. Статистичні дані системи МОЗ. URL: <https://medstat.gov.ua/ukr/statdan.html>
44. Юліш О. І., Гриневич О. Й., Абатуров А. Є., Матяш В. І., Панасюк О. Л., Соломаха Л. М. ФЛАВОЗІД®: специфічна антивірусна активність, клінічна ефективність та безпека застосування при герпесвірусної інфекції у дітей (систематичний огляд) // London Review of Education and Science. – 2015. – №2(18). – Р. 66–89.

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Section 2

2.1. PROTEFLAZID®: SPECIFIC ACTIVITY AGAINST HERPES VIRUS IN PRECLINICAL INVESTIGATIONS AND ITS EFFICACY/SAFETY IN CLINICAL PRACTICE (SYSTEMATIC REVIEW)

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Abstract

It was shown specific antiherpes activity of medicine PROTEFLAZID® in preclinical stage of investigations. It was proved that PROTEFLAZID® has polypharmacologic activity: blocks DNA-polymerase and thymidine kinase in Herpes virus infected cells, induces synthesis of endogenous α - and γ -interferones, has antioxidant activity, and renders apoptotic modulative effect. It was confirmed in clinical conditions ethiopathogenetic efficacy of antiviral medication PROTEFLAZID® (drops) for Herpes virus infection. It was demonstrated safety and therapeutic efficacy of PROTEFLAZID® (drops) in more than 3200 patients (adults, children, pregnant women). Continuous monodirection of therapeutic efficacy of medication was observed in 65 investigations during period since 2000 till 2015.

Key words: PROTEFLAZID®, antiviral efficacy, Herpes virus infection, prophylaxis, treatment.

Introduction

Relevance. Herpesvirus infection (HVI) is a general name for infectious diseases caused by a structurally homogeneous group of viruses belonging to the Herpesviridae family [1]. The family includes more than 100 representatives, of which the most pathogenic for humans are herpes simplex viruses (HSV) types 1 and 2, varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus type 6 (HHV-6), human herpesvirus type 7 (HHV-7), and human herpesvirus type 8 (HHV-8) [1, 2]. The general pathogenetic and biological properties of herpesviruses (HV) include intracellular parasitism, the ability to persist for life in the human body, the dependence of the infectious process on the state of the virus carrier's immunity, and a tendency to relapse. It is the ability of HBV to persist for a long time that determines the possibility of developing several forms of infection in the human body – acute, latent, and chronic recurrent, while at the same time, the transition of one form of infection to another is possible [2].

According to numerous studies, it has been established that from 65 to 90% of the world's population is infected with one or more types of HBV [1, 2, 3]. Human HBV infection is

accompanied by clinical symptoms of the corresponding acute infectious disease in an average of no more than 50% of people. In other patients, the infection is asymptomatic, which is especially characteristic of adolescents and adults. But, despite the initial asymptomatic nature of the clinical manifestations of latent HBV, viruses, even in an inactive, cell-integrated state, can cause chromosomal abnormalities in the human genetic material and, thereby, contribute to the development of carcinogenesis, autoimmune disorders, and dystrophic changes in tissues and organs. HBV are polytropic and during the period of reactivation are capable of affecting almost all organs and systems of the human body. The systemic nature of the lesion is determined by various clinical forms of HIV, the features of which depend not only on the factors listed above (viral virulence, state of the immune system) but also on the type of HVI [2].

The problem of HVI is interdisciplinary. Depending on the predominant area or organ of the lesion, clinical form, age, and gender, patients with HVI can be examined and treated by pediatricians, gynecologists, infectious disease specialists, dermatologists, neurologists, urologists, oncologists, and other specialists. The therapy of HVI is complex and often multi-component. This is due to the fact that currently there is no single drug and treatment regimen that is sufficiently effective against all HSV and the pathological conditions they cause.

According to modern research, flavonoids with antiviral and immunocorrective effects can be used as etiotropic therapy to suppress the reproduction of genital warts.

Since 2000, the arsenal of antiviral drugs has been replenished with the original drug PROTEFLAZID® (drops) ("SMC "Ecopharm" Ltd., Kyiv, Ukraine), which has antiviral, immunomodulatory and apoptosis-modulating effects [4, 5], which was of important practical importance and was accompanied by several scientific studies to assess its effectiveness and safety in herpesvirus pathology.

PROTEFLAZID® is a liquid alcohol extract from wild cereal plants *Deshampsia caespitosa* L. and *Calamagrotis epigeios* L., the main biologically active substance of which is flavonoids similar to quercetin, the basis of the molecule is formed by a flavone oxygen-containing heterocycle.

As it turned out, along with safety, the medicinal product PROTEFLAZID® (drops) is therapeutically effective at any stage of infection (and not only at the time of replication), unlike acyclic nucleosides. In addition, repeated and long courses of the drug did not lead to immunosuppression and did not cause refractoriness of the immune system. The drug confirmed its effectiveness, and effect on various types of HSV and demonstrated the ability to have an anti-relapse effect [4, 6].

The complexity and insufficient effectiveness of the existing therapy of HSV infection became a prerequisite for the analysis of materials confirming the specific antiherpetic activity of the medicinal product PROTEFLAZID®, as well as the pathogenetic justification of the choice, effectiveness, and safety of the drug in the therapy of various clinical forms of HSV in both children and adults in the acute period and during the convalescence period.

Objective of the study

To analyze the scientific and practical evidence base that confirms the effectiveness and safety of the medicinal product PROTEFLAZID® (drops) at the stage of preclinical studies and in clinical practice in the treatment of herpesvirus infections.

Materials and research methods

Scientific publications, reports of preclinical and clinical studies; systematic reviews.

Results and discussion

The systematic review analyzed the results of preclinical and clinical studies of the medicinal product PROTEFLAZID® (drops) in scientific and medical institutions in Ukraine and other countries.

Assessment of the specific antiherpetic activity of the medicinal product PROTEFLAZID® at the preclinical stage of research.

Rybalko S. L. (2002), using Vero cell culture, showed a pronounced antiherpetic activity of the medicinal product PROTEFLAZID®. *In vitro*, with the prophylactic and therapeutic effects of different concentrations of the drug (from 6.8 to 0.212 µg/ml), inhibition of herpes virus reproduction by 3.0–6.0 lg TCD50 was noted [7]. Deryabin P. G. (2013) on a similar cell model (HSV-1 in Vero cell culture) confirmed that PROTEFLAZID® statistically significantly inhibited the cytopathic effect of HSV-1 in the prophylactic and therapeutic exposure schemes at the studied concentrations, and the selectivity index (SI) value was obtained (>100) [8].

In vivo, the antiherpetic activity of PROTEFLAZID® was demonstrated in models of herpetic meningoencephalitis in mice, genital herpes in guinea pigs, and herpetic keratitis in rabbits. The efficacy index (IE) of the preventive effect of the drug was 50.0; the therapeutic effect was 33.3. PROTEFLAZID® at a concentration of 1.36 µg/mL, in a model of genital herpes in guinea pigs, reduced symptoms, the duration of the disease to 9 days, and caused a 60% therapeutic effect. Increasing the concentration to 6.8 µg/mL increased the therapeutic efficacy of the drug to 95%. In a model of herpetic keratitis, treatment with PROTEFLAZID® at a dose of 0.68 µg/mL completely prevents the development of herpetic keratitis in rabbits [7, 9].

In the study of the mechanisms of antiviral action of the medicinal product PROTEFLAZID® Rybalko S. L. (2002, 2010) proves that the active substance of the drug can inhibit virus-specific enzymes in infected cells – thymidine kinase (TK) and DNA polymerase, which leads to a decrease in the ability or complete blocking of virus replication. In the system “brain cells – herpes virus type 1” under the action of different concentrations of the medicinal product PROTEFLAZID® it was shown that the drug at a dose of 10 µg/ml inhibits TK activity by 30%, at a dose of 25–50 µg/ml – by 50%, at a dose of 75–100 µg/ml – by 70%. In the same system, DNA polymerase activity at a concentration of PROTEFLAZID® from 10 to 75–100 µg/ml, decreased from 20 to 28% [7, 11].

Since the medicinal product, PROTEFLAZID® is a multi-component mixture of natural compounds, in order to determine the specific activity of individual compounds, the biologically active substance of the drug PROTEFLAZID® (BAS) was isolated. BAS is presented in the form of stable molecular complex compounds of flavonoid agicones: tricrin, apigenin, luteolin, quercetin, and rhamnosin in the form of O- and C-glycosides (located in the matrix of auxiliary natural substances). The chemical purity of BAS is 93.8%. Rybalko S. L. et al. (2010) conducted a study of the antiviral activity of BAS on a culture of continuous BHK cell line. The authors noted that BAS inhibits the reproduction of the herpes virus in the dilution range from 1:40 to 1:1 280 (concentrations from 12 µg/ml to 0.37 µg/ml). Inhibition of the infectious titer of herpes virus by more than 4.5 lg ID50 was shown. The maximum tolerated concentration of BAS for BHK cells, equal to 825 µg/ml, and the minimum active concentration of BAS

concerning HSV-2, equal to 0.37 µg/ml, allowed us to establish an SI value of 2,230, which indicates a highly selective effect of BAS on HSV-2 [11].

The study of the antiherpetic activity of BAS in *in vivo* experiments was carried out on a model of herpesvirus meningoencephalitis in white mice caused by HSV-1. It was shown that the protective effect of BAS at a dose of 0.48 mg/kg, when administered prophylactically and when administered according to a treatment regimen for IE and inhibition of the infectious titer in the brain tissue of mice was equal to the effectiveness of acyclovir at a dose of 10 mg/kg. Antiherpetic activity (HSV-2) was also studied on a model of genital herpes infection in guinea pigs. The studied drugs (BAS and acyclovir in the form of an ointment) were applied to the scarified infected skin of guinea pigs once a day for 5 days. Experiments have shown that BAS exhibits antiviral activity with a more pronounced therapeutic effect (Treatment Effect Index 93.5%) than acyclovir ointment (Treatment Effect Index 56.0%) [11].

Thus, preclinical studies have shown that BAS PROTEFLAZID® has high SI and IE indices [7–12].

One of the mechanisms of antiviral action of the drug PROTEFLAZID® is interferonogenic activity. Data by Zavelevich M. P. et al. (2002) indicate that the medicinal product *in vitro* dose-dependently stimulates the production of interferon (IFN) in human leukocytes and culture of continuous MDBK cell lines. Typing showed that the drug is an inducer of α - and γ -IFN [13]. Studies have confirmed that PROTEFLAZID® can induce the synthesis of IFN both *in vitro* and *in vivo* as early as 3 hours after administration [10–12].

In addition to antiviral activity, the medicinal product PROTEFLAZID® also exhibits antioxidant properties: it increases the resistance of cells to free radical stress during infections, and reduces the negative effects of drug chemotherapy, which contributes to the adaptation of the body to adverse environmental conditions. *In vitro* experiments have shown that the drug more than doubles the intensity of free radical processes induced by hydrogen peroxide [10].

The medicinal product PROTEFLAZID®, exhibiting apoptosis-modulating activity, contributes to the primary prevention of cancer in the context of chronic (latent) viral infections. The active BAS of the drug, through the activation of caspase 9, restores the ability of cells affected by viruses to apoptosis, reducing the activity of proliferative processes in mutated cells [11].

In 2015, a systematic review was published on the effectiveness of PROTEFLAZID® in Epstein-Barr virus infection in preclinical studies and clinical practice (Hrynevych O. Y. et al., 2015). The review, based on the published results of preclinical and clinical studies, proved the effectiveness of PROTEFLAZID® in this type of herpesvirus infection in children and adults [14].

Therapeutic efficacy and safety of the medicinal product PROTEFLAZID® (drops) in various clinical variants of genital herpes in adults and children. Genital herpes (GH) is a serious medical and social problem. According to foreign researchers, GH ranks 3rd in frequency among sexually transmitted diseases. In most patients, against the background of immunological disorders, GH takes on a chronic recurrent nature, sharply reducing the quality of life of patients, increasing the risk of developing bacterial, neurological disorders, and infertility. Repeated courses of chemotherapeutic antiviral drugs do not guarantee the cessation of GH relapses. The search for new drugs and improvement of existing treatment regimens continues.

In 2000, Ventskiivskiy B. M. presented the results of a clinical study “Open study of the tolerability and preliminary assessment of the efficacy of the medicinal product PROTEFLAZID® (drops) in the treatment of primary and recurrent infection caused by Herpes genitalis”. It was found that PROTEFLAZID® (drops) has high efficacy in the treatment of primary and recurrent HG, and does not cause serious side effects, if compared with the efficacy and tolerability of acyclovir [15].

One of the first was Vovk I. B. et al. (2002), who published data on the effectiveness of the use of the medicinal product PROTEFLAZID® in gynecological practice. Their study involved women with chronic inflammatory processes of the genitals of viral-chlamydial etiology. A significant improvement in the clinical presentation of the disease, normalization of the main factors of the protective function of cervical mucus (sIgA, lysozyme, and C₃-component of complement), and a decrease in the seeding of the genital tract with pathogenic microflora were noted [16].

Andriets O. A. et al. (2004, 2005) studied the role of HV in the development of inflammatory processes of the genital organs and the therapeutic features of these diseases in girls of prepubertal and pubertal age. PROTEFLAZID® was prescribed only to those girls in whom HSV2 antigen was detected in smears, for a course of up to 4 weeks. It was found that after 1 week of taking the drug, clinical manifestations and complaints significantly decreased in 40% of patients, and after 2 weeks of taking the drug, rashes on the genitals and subjective sensations completely disappeared in 85% of patients. During the therapy, an increase in the level of IL-1β, IL-2, TNF-α in peripheral blood and IL-1β, sIgA in vaginal secretion was observed, this effect persisted after the course of therapy, which indicated the process of recovery and repair [17, 18].

During this period, Kyshakevich I. T., Romashchenko O. V. et al. (2005) substantiated the feasibility of prescribing and confirmed the effectiveness of the medicinal product PROTEFLAZID® in the treatment of inflammatory diseases of the genitals, including the diseases on the background of mixed infections. As a result of the studies, similar positive data were obtained. The drug caused positive changes in local immunity – the levels of immunoglobulins and lysozyme in cervical mucus normalized, sIgA levels significantly increased with a parallel decrease in the level of IgM, which indicated the activation of humoral immunity and a decrease in the antigenic load, due to a decrease in genital infection. Some patients experienced an exacerbation in the first days of taking the drug, which then quickly subsided. Positive dynamics were noted in more than 83.3% of patients. The frequency of relapses decreased to 5.0% [19, 20].

In 2003–2005, Grinkevich T. M. conducted a series of studies to evaluate the effectiveness of various GH therapy regimens. It was confirmed that PROTEFLAZID® eliminates the imbalance of cellular and humoral immunity, has a positive clinical effect, reduces the frequency of relapses by 19%, contributes to the normalization of the vaginal microbiocenosis, and accelerates re-epithelialization in herpetic endocervicitis [21, 22].

Another author (Gopchuk O. M., 2006) developed complex therapeutic and preventive measures for the correction of neurohumoral disorders of the menstrual cycle in patients with HV based on the medicinal product PROTEFLAZID®. Against the background of therapy, he observed the normalization of the menstrual cycle, the absence of HV detection in specific studies, and the restoration of the vaginal microbiocenosis [23]. Continuing the research, Gerasimova T. V., and Gopchuk O. M., in 2007 presented the results of the use of

the medicinal product PROTEFLAZID® in the treatment of HV and papillomavirus infection. PROTEFLAZID® was prescribed in combination with Viferon. The duration of anti-relapse therapy was 3–6 months. The clinical effectiveness of the proposed treatment regimen was 82.8%, and the frequency of recurrence of herpetic infection decreased by 2–3 times, HPV – by 1.5–2 times [24].

Zapolskiy M. E. in publications of 2006 and 2012 presented data on clinical features and therapeutic efficacy of the medicinal product PROTEFLAZID® in patients with HG complicated by exudative erythema multiforme (EEM). It was noted that in cases of uncomplicated GH, positive dynamics, against the background of therapy, is noted already on the 4th–5th day of treatment, complete disappearance of manifestations – on the 5th–6th day. In cases of EEM development, epithelialization of erosions and bullous elements in 96.9% of patients of the main group was completed by the 9th–10th day of treatment. Repeated relapses of EEM during the year were noted only in 12.5% of patients. In immunological indicators, positive dynamics were noted regarding the main elements of cellular immunity, a decrease in the content of circulating immune complexes (CIC) [25, 26].

Similar studies were conducted by other authors. In particular, Lisovyi V. M., Yakovleva O. V. (2006), Shymanskaya I. G. (2007) evaluated the effectiveness of the medicinal product PROTEFLAZID® in the complex therapy of patients with GH, in particular, both in clinically pronounced and asymptomatic forms. It was noted that while taking the drug, 95% of patients had a positive clinical outcome; 28.1% of patients noted a reduction in the duration and activity of clinical manifestations of relapses [27, 28].

According to Aizyatulov R. F. (2008), during the use of the medicinal product PROTEFLAZID® in the treatment of mixed urogenital infection in men, positive clinical dynamics were observed already on the 3rd–5th day of therapy; on the 6th–8th day, discharge stopped, and elimination of the pathogen was observed in most patients [29].

Flaks G. A. (2008) confirmed the high effectiveness of the medicinal product PROTEFLAZID® in patients with HVI, in the form of a decrease in the frequency of exacerbations, normalization of laboratory indicators (disappearance of the virus in PCR smears, decrease and/or disappearance of antibody titer in ELISA) in the acute period of GH manifestations and, what is especially important, during remission [30].

Popova T. V. (2008) studied the features of differentiated treatment regimens for women (including HIV-infected women) with inflammatory diseases of the cervix. 68 HIV-infected women were prescribed PROTEFLAZID® (intramuscularly and topically) as part of a prolonged therapy program. It was found that the overall efficacy of this course of therapy was 93.8%, with most patients experiencing a sustained regression of inflammatory symptoms and normalization of vaginal microflora [31].

At the same time, researchers Sundukov A. V. et al. (2008) conducted a clinical trial of PROTEFLAZID® in the complex treatment of some STIs in adults. The study included 75 patients, who were divided into 4 groups depending on nosology (genital herpes – 22 patients, herpes zoster – 18 patients; herpes simplex – 19; infectious mononucleosis (IM) – 2 patients). It was proven that the drug has good tolerability and minimal side effects. In the treatment of GG, the drug is highly effective, reducing the number of relapses by 8.2–8.4 times, and increasing the interrelapse period by more than 2.5 times. In the treatment of herpes zoster, the maximum therapeutic efficacy was noted in the combination of the medicinal product PROTEFLAZID® with acyclovir. In the treatment of herpes simplex, the

effectiveness of the drug is comparable to acyclovir. In the treatment of IM, the effectiveness of the drug was also noted, which was manifested in positive clinical dynamics and stabilization of hematological parameters [6].

Isakov V. A., Ermolenko D. K. (2009) conducted an open controlled clinical study to determine the effectiveness of the medicinal product PROTEFLAZID® in the treatment of patients with recurrent GH. The high clinical effectiveness of the drug was proven: the frequency of relapses decreased by 7.5 times, 32% of patients had no relapses during the year. The average interrelapse period increased by 2.8 times. Positive dynamics were also noted in immunological indicators. After a course of treatment, by direct immunofluorescence, herpes viruses were not detected in 92% of patients for 6 months [32].

In 2011, Romanyuk M. G. et al. conducted a comprehensive study to evaluate the characteristics of the therapy of patients with GH. Against the background of therapy, positive kinetic dynamics, and epithelialization were observed on average on the 6th day of treatment. The average number of relapses in patients decreased from 6 times per year to 3. The authors compared the effectiveness of the medicinal product PROTEFLAZID® with the effectiveness of famciclovir, but the frequency of side effects with the drug PROTEFLAZID® was significantly lower [33].

In the same year, researchers Baev A. I. et al. (2011) published a series of reports on the results of studies of clinical and pathogenetic features and differentiated approaches to the therapy of mixed sexually transmitted infections (STIs). According to the results of their observations, it was established that the use of the medicinal product PROTEFLAZID® in the complex treatment of mixed STIs is an effective and safe method of therapy, which was confirmed by clinical improvement, normalization of immunological and biochemical indicators, the absence of side effects and adverse reactions [34].

Further studies by Ryzhko P. P., Roschenyuk L. V. (2012), Klymenko P. M. et al. (2012), Rak L. M., Yuzko O. M. (2013) also noted positive dynamics when using the medicinal product PROTEFLAZID® as regression of clinical manifestations, stable therapeutic response and a 1.5-fold reduction in the frequency of GH relapses with a reduction in their duration [35–37].

In 2015, Kornatskaya A. G. [38] and Benyuk V. O. [39] presented the results of two independent clinical studies on the comparative assessment of the efficacy and tolerability of the medicinal products PROTEFLAZID® (suppositories) and PROTEFLAZID® (drops, in the form of vaginal tampons) in patients with herpetic and urogenital viral-bacterial infection.

In the study by Kornatskaya A. G. (2015), 70 women with a verified diagnosis of genital herpes (HSV-1, HSV-2) in the acute stage participated. Patients were divided into the main (n=35) and control (n=35) groups based on the simple randomization method. Patients in the control group received the reference drug PROTEFLAZID® (drops) in the form of vaginal tampons with a solution of the drug. By the end of the treatment course and during the 8-week observation period, there was a significant increase in the level of local immunity indicators (secretory IgA, lysozyme, C₃-component of complement) compared to the baseline. In particular, the level of secretory IgA increased by the 10th day of treatment, remaining significantly high during the 8-week observation period (from 990.45 to 1,825.24 µg/l); the level of lysozyme increased by the 10th day of treatment, remaining significantly high during the 8-week observation period (from 28.39 to 41.31 µg/l); Complement C₃ levels increased by day 10 of

treatment and returned to baseline by the end of the 8-week follow-up period (from 17.68 µg/g protein at screening to 81.17 µg/g protein at day 10 and 20.37 µg/g protein at week 8). After the completion of the 10-day course of treatment and the 8-week follow-up period, there was a decrease in HSV DNA viral load and a significant decrease in HSV markers (Ig G, Ig M) compared to baseline [38].

All women who participated in the study observed relief of clinical manifestations of genital herpes, the effectiveness of treatment with the medicinal product PROTEFLAZID® (drops) was 100%, and there were no recurrences of herpes infection. During the study, it was shown that the drug PROTEFLAZID® (drops) has high efficacy and good tolerability, no serious or unexpected adverse reactions were noted during the treatment, and laboratory parameters did not reveal any negative changes [38].

The same results were shown by Benyuk V. O. (2015) in a comparative clinical study involving women with urogenital viral-bacterial infection [39].

However, the problem of chronic recurrent urogenital infection in adults should be considered not only from the perspective of the complexity of therapy but also taking into account its adverse impact on conception, pregnancy, and the fetus.

Thus, according to modern literature [40], one of the most common causes of maternal and perinatal morbidity and mortality of the fetus is intraamniotic infection, in particular, various forms of HVI. The main complications in patients with HVI are the threat of abortion, polyhydramnios, chronic fetal hypoxia, and during childbirth – primarily temporary rupture of the membranes and abnormalities of labor activity. The highest frequency of the threat of abortion is in the recurrent form of herpetic infection (73.3%). The development of infection is facilitated by the suppression of cellular and humoral immunity during pregnancy, caused by physiological immunosuppression due to a decrease in the number and activity of T- and B-lymphocytes, increased production of corticosteroids, estrogens, and progesterone. Primary HVI leads to spontaneous abortion in early pregnancy, the occurrence of developmental anomalies in the fetus, and after 20 weeks is one of the main causes of premature birth and fetoplacental insufficiency [40, 41].

Timely prevention of recurrences of HVI in patients at risk is a priority direction, however, the use of toxic antiviral drugs during pregnancy has limitations. However, the medicinal product PROTEFLAZID®, according to preclinical studies, has a high degree of safety and therefore was approved for use in pregnant women and children.

Vdovychenko Yu. P. et al. (2003) were among the first to conduct comprehensive studies of the therapeutic and prophylactic efficacy of the medicinal product PROTEFLAZID® in pregnant patients. The drug was taken by women with latent and recurrent forms of HVI. The duration of the course was 21 days. In the latent form of herpetic infection, 1 course of the medicinal product PROTEFLAZID® was prescribed, and in the case of recurrent – from 2 to 3 courses. Women were monitored throughout the pregnancy, starting from 16–17 weeks and until delivery with an interval of 2–3 weeks. It was found that therapy, which included PROTEFLAZID®, allowed reducing the frequency of threatened abortion (from 73.3 to 36.7%), fetoplacental insufficiency (from 46.7 to 23.3%), preeclampsia of varying severity (from 23.3 to 10.0%), premature birth (from 16.7 to 6.7%), premature rupture of membranes (from 46.7 to 20.0%) and labor abnormalities (from 26.7 to 10.0%). In newborns, a decrease in the frequency of asphyxia of varying severity (from 43.3 to 23.3%), manifestations of intraamniotic infection (from 23.3 to 10.0%), and the absence of generalized infection were noted [42].

Simrok V. V., Gordienko O. V. (2003) studied the effectiveness of the medicinal product PROTEFLAZID® in the treatment of viral infection in 34 (20 cases of GI and 14 – CMVI) women of childbearing age with a history of perinatal losses. It was established that PROTEFLAZID® had a good clinical and immunological effect. Pregnancy proceeded with a significantly smaller number of complications, in particular the development of fetoplacental insufficiency, and usually ended with timely delivery with the birth of fetuses in satisfactory condition [43].

Nagorna N. F., Nikolaeva S. V. (2006, 2007) performed a series of studies to study the therapeutic, prophylactic, and immunomodulatory efficacy of the drug PROTEFLAZID® both in monotherapy and in combination with acyclovir in patients with genital herpes who suffer from miscarriage. It was found that differentiated pre-pregnancy preparation and therapy during pregnancy with the inclusion of the medicinal product PROTEFLAZID® helped to reduce recurrences of herpetic infection and the risk of perinatal loss by 35%. The duration of recurrence was reduced by 1.6–2 times, and the frequency of recurrences decreased to 1–2 cases in 8 months. Immunological indicators showed an increase in the total number of T- and B-lymphocytes, the population composition of T-cells normalized, and the concentration of CIC decreased [44–47].

The data of Dolgov G. V., Abashin V. G. (2009) confirm the feasibility and effectiveness of prescribing the medicinal product PROTEFLAZID® to couples of reproductive age with a history of pregnancy loss [48].

Benyuk V. O. et al. (2012) conducted an analysis of the prevention and treatment of HVI in pregnant women with metabolic syndrome. 30 patients received PROTEFLAZID® according to the basic regimen in the pre-pregnancy period (for 3–6 months) and during pregnancy. Against the background of taking the drug, a significant decrease in clinical manifestations and a reduction in the terms of HVI recurrence (more than 1.4 times) were noted. Positive dynamics in immunological indicators were determined, and obstetric and perinatal pathology was registered less often (by 50%). The frequency of early pregnancy termination decreased from 73.3% to 36.7%, fetoplacental insufficiency from 46.7 to 23.3%. The use of the drug allowed halving the frequency of pathology in newborns [49].

Similar positive clinical and immunological results of the use of the medicinal product PROTEFLAZID® in pregnant women, in active viral-bacterial mixed infection to reduce perinatal pathology, were obtained by Azimova E. I. et al. (2011) and Reznichenko N. A. (2013) [50, 51].

In cases of lack of adequate therapy and monitoring of intrauterine infections, the prognosis for the life of newborns with HVI is extremely unfavorable. Mortality with congenital HVI in newborns can reach 30%, and in surviving children, severe lesions of the nervous system and multiple organ failure are noted. If children are infected with HSV after birth, the clinical symptoms of the corresponding acute infectious disease are nonspecific and diverse: erythema multiforme (HIV-6), aphthous stomatitis – “thrush” (HSV-1, HSV-2), chickenpox (varicella), infectious mononucleosis (IM) (EBV); and mononucleosis-like syndrome (CMV). In children, the infection process is often asymptomatic, but the ability of viruses to persist for a long time can potentiate the development of pathological processes in various organs and systems: bronchopulmonary, cardiovascular, nervous, immune, etc. for several years. In this regard, the physiological and mental harmony of the child’s development depends on the timeliness and adequacy of the appointment of antiviral therapy.

PROTEFLAZID®, due to its high safety, is the drug of choice in the treatment of HVI in children, starting from birth [2, 41, 52].

Kriuchko T. A., and Nesina I. M. et al. (2002) were among the first to study the effectiveness of the medicinal product PROTEFLAZID® in the treatment of neuroinfections in 20 children. The drug was prescribed to children in age-appropriate dosages for up to 4 months. On the background of therapy, patients noted a pronounced and stable effect in the dynamics of clinical and laboratory indicators: a decrease in intoxication phenomena, improvement in general well-being, rapid elimination of meningeal syndrome, and correction of immunological disorders [52].

This research was continued by Yershova I. B., Goncharova T. A., Skorodumova N. P. (2003). The authors noted that the use of a combination of exogenous interferon alfa-2b and the medicinal product PROTEFLAZID® helps prevent relapses and accelerates the functional activity of children with viral meningoencephalitis [53].

According to Ovcharenko L. S. et al. (2004, 2006), in children with cytomegalovirus and chlamydial infections, as well as frequent ARVI, the use of the medicinal product PROTEFLAZID® allows to reduce the number of bacterial complications of ARVI and contributes to the correction of immunological indicators, which is associated with the eradication of pathogenic microflora [54, 55].

Usachiova O. V. et al. (2005) performed a series of studies to assess the clinical efficacy and tolerability of the drug PROTEFLAZID® in the treatment of intrauterine CMV infection in 23 children of the first year of life and infectious mononucleosis (IM) in 38 children. Against the background of therapy in children with IM, recovery was achieved in 35.3%. In 78.5%, a gradual decrease in the manifestations of hepatomegaly was noted, and the ALT level dynamically decreased. In patients with CMV infection, the leveling of cytolytic syndrome, positive dynamics in neurological status, and inhibition of viral replication were noted [41, 58].

In 2005, Chernyshova O. E. published data on the study of the effectiveness of the medicinal product PROTEFLAZID® in the treatment of children with HVI. It was shown that against the background of treatment, acute manifestations of infection were stopped in 67% of patients, in 64% – it was possible to translate the chronic recurrent course of HVI into latent, the number and duration of ARVI and the frequency of complications decreased by 2.5 times [57]. Further, continuing the study, the author noted the high effectiveness of the drug against EBVI [58].

The combined treatment regimen for CMV infection (a combination of Viferon and PROTEFLAZID®) was studied by Turlibekova S. S. (2006). 48 children under the age of one year were under her supervision. During the combined therapy, positive dynamics of all clinical symptoms with complete elimination of viruses were noted in children on the 12th day [59].

Nagorna N. V., Vynogradov K. V. in 2007, on the basis of the Department of Pediatric Cardiac Surgery of the V. K. Gusak Institute of Emergency and Reconstructive Surgery of the National Academy of Medical Sciences of Ukraine, studied the effectiveness of the medicinal product PROTEFLAZID® in 27 children with congenital heart defects (CHD) with different courses of HVI. The duration of observation was 12 months. According to the results of the study, 3 and 12 months after the end of treatment, the children had no laboratory markers of the active course of HVI, and the titers of specific IgG decreased. Acute respiratory diseases and bronchitis were registered less often [46]. The therapeutic effectiveness of the drug PROTEFLAZID® in children with recurrent obstructive bronchitis on the background of recurrent HVI was shown by the studies of Shamsieva F. M., Mirsalikhov N. Kh. et al. (2011) [60].

Biletska G. A. et al. (2011) also studied the effectiveness of the medicinal product PROTEFLAZID® in the treatment of IM in 72 children. Their data confirm that the use of the drug in the complex therapy of children with IM reduces the duration of manifestation of the disease and shortens the children's hospital stay, and also reduces the possibility of relapses [61].

In 2013, Znamenska T. K. et al. published data on the unidirectional positive therapeutic efficacy of the medicinal product PROTEFLAZID® in the treatment of intrauterine infection in newborns of mothers with viral-bacterial infection. The drug was prescribed for a month. Against the background of therapy, the time spent in intensive care and the use of artificial lung ventilation was significantly reduced, jaundice regressed faster, neurosonographic changes (ventriculodilation) were less pronounced, and intracerebral hemodynamics improved [40].

According to the results of the presented studies, it can be concluded that in pediatric practice of treatment of HVI, the medicinal product PROTEFLAZID® (drops) has high therapeutic efficacy.

As is known, HV has high tropism to the epithelium of the oral mucosa and nose, and therefore it can cause both acute and chronic recurrent gingivostomatitis, cheilitis, and pharyngitis, the therapy of which must be carried out taking into account the viral component of the disease.

Yakovets V. V., Belichenko Yu. M. (2004) studied the effectiveness of the medicinal product PROTEFLAZID® in the treatment of herpetic lesions of the oral mucosa. Patients with gingivostomatitis had their oral mucosa treated with the drug PROTEFLAZID®. Positive clinical dynamics were observed from the first days of therapy, aphthous epithelization – on the 3rd day, and recovery on the 6th day of treatment. The appearance of repeated rashes in the acute period was recorded in only 8% of patients. With prolonged use of the drug, a decrease in the frequency of repeated relapses was observed [62]. The effectiveness of the medicinal product PROTEFLAZID® in the treatment of herpetic gingivostomatitis in young children was confirmed by data obtained in studies conducted by Gerasimov S. V. et al. (2006) [63].

In the large structure of neuroinfections, HVIs of the nervous system occupy a special place. In terms of severity, diversity, and features of clinical manifestations of HV neuroinfections can vary from mild subclinical forms to severe necrotizing encephalitis, accompanied by high mortality (up to 80%). HV eye damage (herpetic keratitis, HK) can be an isolated manifestation of HVI and can precede the development of severe encephalitis. HK often recurs and causes severe complications, such as corneal opacification, secondary glaucoma, cataracts, etc. The outcome of HVI damage to the nervous system and eyes, in these cases, depends on timely and adequate antiviral therapy both in the acute period and during convalescence [2]. The first studies on improving antiviral therapy regimens based on the medicinal product PROTEFLAZID® in patients with HV lesions of the nervous system were performed by Matyash V. I. et al. (2002). The authors proposed a two-stage therapy: stage 1 – selective antiviral therapy: acyclic nucleosides (up to 12 days) and PROTEFLAZID® (up to 3 months); stage 2 – long-term restorative therapy with the use of efferent therapy. It was established that the proposed regimens allowed for reducing the required dose of acyclovir by 2 times (relative to the recommended one) and achieving a stable restoring function of the affected organs and systems [64].

The effectiveness and feasibility of using the medicinal product PROTEFLAZID® in the treatment of HK were among the first to be substantiated in 2003 by Petrunia A. M., Vorotnikov S. V. The course of HK treatment with the drug PROTEFLAZID® was repeated if necessary 2–3 times a year. Against the background of therapy, the duration of corneal syndrome was reduced, compared with the comparison group, by an average of 3.5 ± 0.6 days, pericorneal injection – by 3.1 ± 0.8 days, corneal edema – by 2.8 ± 0.4 days. An increase in visual acuity after treatment in the main group was noted in 77.3% of patients. The development of purulent-inflammatory complications of HK in the group taking the drug was not observed. During dispensary follow-up during the year, relapses of HK in the main group were observed in 9.1% of patients [65].

In ophthalmological practice, the data of researchers Rykov S. O., Znamenskaya M. A. (2010), and Kamilov H. M. et al. (2011) are presented, which confirmed the previously mentioned effectiveness of the medicinal product PROTEFLAZID® in the treatment of recurrent HK. The authors found that the inclusion of the drug in the HK therapy contributes to a faster reduction in the intensity of corneal syndrome, discharge in the conjunctival cavity, eyeball injection, corneal edema, and inflammatory infiltration. Lysis of precipitates was observed within 1–1.5 months. The recurrence rate decreased to 5%, compared to 16.6% in those receiving traditional therapy [66, 67].

The particulars of the effect of the medicinal product PROTEFLAZID® on the immune and interferon status of patients with HB lesions of the NS were described in the publication by Goshko (Panasiuk) O. L. et al. (2005). It was shown that against the background of taking the drug, a more pronounced tendency to normalize the cellular link of immunity is noted (an increase in the total number of lymphocytes (CD3), natural killers (CD16), functional activity of mononuclear cells and neutrophils). The dynamics of induction of interferon formation during treatment was characterized by the prevalence of γ -IFN activity from the 2nd to the 9th day of taking the drug, with a further increase in α -IFN on the 10th day. The time of interferon conversion coincided with the onset of positive clinical dynamics. Against the background of long-term (up to 3 months) daily use of the drug, inhibition of α - and γ -IFN activity was not observed, which indicates the absence of refractoriness of immunotropic cells to IFN induction [68]. A year later, data were published by Panasiuk O. L. (2006), confirming the effectiveness of etiopathogenetic therapy with the medicinal product PROTEFLAZID® in patients with HV lesions of the nervous system. It was shown that PROTEFLAZID® in monotherapy of moderate and uncomplicated forms of HVI had moderate clinical and immunological efficacy, which was manifested by regression of somato-neurological symptoms on the $8\text{th} \pm 2.6\text{th}$ day of treatment; seroconversion of specific antiviral antibodies on the $2\text{nd} \pm 0.8\text{th}$ month of treatment; an increase in the number of NK cells by 17%, functional activity of mononuclear cells by 20%, neutrophils by 10% and a decrease in the severity of neuroautoimmune reactions by 1.5 times. The use of the drug during convalescence allowed to reduce the frequency of repeated clinical and virological relapses (virus “+”) by 1.9 times, clinical (virus “-”) relapses – by 1.3 times, as well as to reduce the severity of the disease and accelerate the achievement of a therapeutic effect during repeated therapy of relapse [69].

Chopiak V. V., Potiomkina G. O. (2008) studied the effect of the medicinal product PROTEFLAZID® on the clinical course, immunological and virological parameters of patients with chronic EBV infection in adults at the reactivation stage.

During the study, it was found that in 80% of patients, the use of the drug contributed to a significant improvement in general well-being. In immunological indicators, an increase in the mass of the killing subpopulation of lymphocytes due to T-cytotoxic lymphocytes, the functional ability of NK cells to synthesize interferons was observed, the suppressive activity of regulatory CD4+/CD25+ lymphocytes decreased, and acquired and innate antiviral immunity was activated [70].

Thus, the presented research results indicate the pathogenetic validity and effectiveness of the medicinal product PROTEFLAZID® (drops) in the therapy of HV lesions of the nervous system and eyes.

As is known, HVI plays an important role in the development of various somatic pathologies, both as a result of the direct effect of viruses on organs and tissues and indirectly, through the development of immunological disorders. In this regard, the medicinal product PROTEFLAZID® with immunomodulatory and antiviral purposes can potentiate the effect of complex therapy of the specified category of patients.

Sydorenko E. V., Dryanska V. E. (2007, 2010) conducted a study to study the clinical and microbiological (60 patients) and immunological (25 patients) effects of the medicinal product PROTEFLAZID® (drops) in patients with chronic uncomplicated pyelonephritis, in whose blood a diagnostic increase in antibody titers (IgG) to Herpes simplex virus, Cytomegalovirus, *Toxoplasma gondii* and/or *Chlamydia trachomatis* was detected. A significant decrease in the content of pro-inflammatory cytokines in the blood and urine was noted, indicating a more active suppression of inflammation. The indicators of the lipid peroxidation system of antioxidant defense (LP-AOD) were normalized, a decrease in the frequency of relapses was clinically observed, and renal dysfunction was prevented [71, 72].

The effectiveness of PROTEFLAZID® and Viferon in children with hematuric forms of chronic pyelonephritis and concomitant chronic EBV infection was studied by Borisova T. P., Tolchennikova O. M. (2013). It was noted that during treatment in most patients, signs of viral activity were eliminated, the frequency of hematuria registration decreased, the functional state of the kidneys improved, correction of interferon status was observed, the level of α -IFN increased 2.7 times, the level of γ -IFN increased 3.6 times with a decrease in the level of pro-inflammatory cytokines [73].

Kolesnyk M. O. et al. (2014) continued research in this direction. Adult patients with pyelonephritis were under their supervision. Some patients received PROTEFLAZID® in addition to antibacterial therapy. It has been established that PROTEFLAZID® contributes to the normalization of cytokine levels in the blood and urine; and ensures the eradication of pathogens (especially herpes simplex virus and ureaplasma) [74].

As can be seen from the above results, the medicinal product PROTEFLAZID® (drops) is widely used by practicing physicians and is included in various treatment and prevention regimens for herpesvirus and viral-bacterial infections in children and adults. Since the approval of the medicinal product PROTEFLAZID® in clinical practice, over the past 15 years, more than 65 clinical studies and observations have been performed to study the efficacy and safety of the drug against herpesvirus and viral-bacterial infections, involving more than 3,200 patients who took the drug PROTEFLAZID® (drops), including pregnant women and children. The main results of these observations are presented in the table.

Table. Main results of clinical studies of the efficacy and safety of the medicinal product PROTEFLAZID® (drops) in children and adults during 2000–2015.

No.	Authors, year, source	Number of patients		Main results of the medicinal product PROTEFLAZID® use in clinical practice
		Total	Taking the product	
1	Venzkivskiy B.M., 2000 [15]	60 adults	40	PROTEFLAZID® in the treatment of primary and recurrent infection caused by Herpes genitalis is characterized by high efficiency, in the absence of serious side effects. Complete elimination of clinical manifestations of the disease by 5–7 days of treatment was noted in 60% of patients, and cure in all patients occurred by 10 days of drug use.
2	Vovk I.B. et al., 2002 [16]	68 adults	68	Normalization of the main factors of the protective function of cervical mucus (sIgA, immunoglobulins, lysozyme, and C ₃ -component of complement), reduction of the colonization of the genital tract with microflora, which has a beneficial effect on the state of local immunity of the female genital tract as a whole, was noted. In 85.3% of women, according to PCR, the virus was not detected, in 91.2% there was no Ig.
3	Kriuchko T.A. et al., 2002, [52]	20 children	20	There was a decrease in intoxication, improvement in well-being, rapid cessation of meningeal syndrome, restoration of the CD4+/CD8+ ratio (1.3 ± 0.32), increase of immunoglobulins in the blood serum Tg G (8.4 ± 1.8); Tg A (3.3 ± 0.05); Tg M (0.4 ± 0.03)
4	Matyash V.I. et al., 2002 [64]	25 adults	25	PROTEFLAZID® with efferent treatment methods allows you to reduce the required dose of acyclovir by half (relative to the recommended one) and achieve a stable effect on restoring the function of the affected organs and systems.
5	Vdovychenko Yu.P. et al., 2003 [42]	120 pregnant females	30	PROTEFLAZID® allows to significantly reduce the frequency of threatened abortion (from 73.3 to 36.7%), fetoplacental insufficiency (from 46.7 to 23.3%), preeclampsia of varying severity (from 23.3 to 10.0%), premature birth (from 16.7 to 6.7%), premature rupture of membranes (from 46.7 to 20.0%) and labor abnormalities (from 26.7 to 10.0%).
6	Grinkevich T.M., 2003 [21]	20 females	20	PROTEFLAZID® alleviates the course of the disease, reduces the frequency of relapses, and extends the interrelapse period. It increases the phagocytic activity of neutrophils and stabilizes the cellular component of immunity.
7	Yershova I.B. et al., 2003 [53]	86 children	86	Reduction of the frequency of relapses, and complications, and shortening the recovery period for children after meningoencephalitis.

8	Lutsyk B.D. et al., 2003 [75]	25 adults	25	After the treatment, clinical healing of cervical erosion was noted in 15 patients out of 25. In all 35 patients, only individual cells infected with viruses were found after treatment. In 7 patients, symptoms of chronic fatigue and discomfort in the lower abdomen, which often occurred before menstruation, disappeared.
9	Petrunia A.M., Vorotnikov S.V., 2003 [65]	47 adults	22	During the treatment process, patients in the main group demonstrated positive dynamics of clinical indicators. In the main group, the duration of corneal syndrome was reduced on average by 3.5 ± 0.6 days compared to the reference group ($P < 0.05$), pericorneal injection – by 3.1 ± 0.8 days ($P < 0.05$), corneal edema – by 2.8 ± 0.4 days ($P < 0.05$).
10	Simrok V.V., Gordienko O.V., 2003 [43]	34 adults	19	PROTEFLAZID® in women in the complex treatment of HVI and CMVI before pregnancy contributed to the stabilization of the level of antibodies to specific IgG at the level of norm in the vast majority of the examined (in 8 pregnant women out of 11 patients) on the background of the absence of IgM in all 11 pregnant women. All examined pregnant women of the main group gave birth at term with fetuses with an average weight of $3,340 \pm 55$ g, compared to the comparison group of $2,096 \pm 30$ g.
11	Fedotov V.P. et al., 2003 [76]	34 adults	34	Already on the 2nd-3rd day of treatment, the development of new rashes stopped; there was a tendency towards epithelialization of erosions and a decrease in the severity of subjective sensations. Complete resolution of clinical manifestations of the disease when using PROTEFLAZID® was noted on the 5th-7th day of taking the drug in 23 (69.1%) of the observed patients, on the 8th-9th day – in 11 (30.9%). Clinical recovery was noted in 25 (70.9%) patients whose treatment complex included PROTEFLAZID®, a significant improvement in 9 (29.1%). No effect or worsening of the course of the disease when using PROTEFLAZID® was noted.
12	Shvediuk S.V., 2003 [77]	22 adults	11	In the group of patients who received PROTEFLAZID® simultaneously with complex therapy, a positive clinical presentation was observed in 81.8% (in the control group – 72.7%), a stable microbiological effect of chlamydia cure was in 90.9% (versus 63.6% in the control group), and the frequency of recurrence of herpetic rashes was noted in 18.2% of the observed. No side effects of the drug, both locally and internally, were detected.
13	Andriets O.A. et al., 2004 [17]	20 children	10	After the 1st week of taking the drug, 40% of patients had significantly reduced clinical manifestations and complaints, and after 2 weeks of taking the drug, in 85% of patients, genital rashes and subjective sensations disappeared completely.

14	Ovcharenko L.S. et al., 2004 [54]	60 children	60	PROTEFLAZID® is active against intracellular pathogens (cytomegalovirus, chlamydia), which makes it possible to recommend it for the treatment of children with both acute and chronic forms of this pathology. A repeated course of treatment of resistant forms after 1.5–2 months allows for achieving normalization of laboratory indicators in 100% of cases.
15	Kyshakevych I.T., 2004 [19]	170 adults	31	The levels of immunoglobulins and lysozyme in cervical mucus were normalized, and SIgA levels significantly increased with a parallel decrease in IgM levels, indicating activation of humoral immunity and a decrease in antigenic load due to a decrease in genital infection.
16	Nikiforova T.O. et al., 2004 [78]	26 adults	14	The medicinal product PROTEFLAZID® has a pronounced effectiveness in the treatment of patients with EBV infection. When it is used, intoxication and lymphoproliferative phenomena disappear more quickly, liver function is restored, and the level of IgG in the blood is significantly reduced. Increased body temperature and general condition normalized in 76.8% of patients receiving the drug, compared to 50.0% of the control group. A decrease in lymphadenopathy was noted in 35.7% of patients compared to 25.0% of the control group.
17	Andriets O.A., 2005 [18]	279 children	42	The levels of IL-1 β , IL-2, TNF- α in peripheral blood and IL-1 β , sIgA in vaginal secretion during treatment and after the course of therapy increased, indicating the process of recovery and repair.
18	Gley A.I., 2005 [79]	47 adults	27	Clear positive dynamics were described as a rapid decrease in the size of the lymph nodes and spleen, improvement in hemogram indicators (decrease in the number of leukocytes, disappearance or decrease in the number of atypical mononuclear cells), and biochemical indicators (normalization or decrease in transaminase activity). After 2 months, viremia was noted in 48% of patients taking the drug and in 98% in the control group.
19	Romashchenko O.V., Rudenko A.V., 2005 [80]	60 females	30	PROTEFLAZID® in the complex therapy of inflammatory diseases of the genitals caused by mixed infection has higher clinical, microbiological, and immunological effectiveness, if compared with traditional methods of treatment of this pathology. The frequency of recurrence of the disease during basic therapy was 16.6%, and when using the drug PROTEFLAZID® – 5.0% ($p < 0.05$)

20	Usachiova O.V. et al., 2005 [81]	38 children	17	When using the medicinal product PROTEFLAZID®, recovery was achieved in 35.3% (in the control group – 19.1%). In 78.5% of children who took the drug, a gradual decrease in hepatomegaly was noted, and in 75% of children with cytolytic syndrome, the level of ALT decreased dynamically.
21	Usachiova O.V., 2005 [41]	23 children	23	On the background of treatment with PROTEFLAZID®, in all cases, regardless of the clinical form of the disease, a positive serological result was achieved. This was manifested in the disappearance of immunoglobulins M against CMV and a decrease in the content of anti-CMV IgG. Positive dynamics were achieved in children with intrauterine CMV infection in terms of clinical symptoms of the disease and stabilization of specific immunological indicators.
22	Chernyshova O.E., 2005 [57]	199 children	199	On the background of the treatment, it was possible to stop the acute manifestations of the infection in 67% of patients and to convert the chronic recurrent course of herpesvirus infection into latent – in 64%. In all observed children, the number and duration of recurrent respiratory diseases and the frequency of complications decreased by 2.5 times.
23	Goshko O.L. et al., 2005 [68]	68 adults	35	In patients of the main group with PROTEFLAZID®, there was a more pronounced tendency towards normalization of the cellular component of immunity (increased total number of lymphocytes (CD3), natural killers (CD16), functional activity of mononuclear cells and neutrophils). The dynamics of induction of interferon production was characterized by the prevalence of γ -IFN activity from 2 to 9 days of taking the drug, with a subsequent increase in α -IFN on day 10. The time of interferon conversion coincided with the onset of positive clinical dynamics.
24	Grinkevich T.M., 2005 [21]	120 adults	49	With the treatment, the imbalance between the cellular and humoral links of immunity was eliminated, a positive clinical effect was noted in 82.7% of cases, a decrease in the frequency of relapses by 19%; the vaginal microbiocenosis in 83.6% of women normalized, re-epithelialization in case of herpetic endocervicitis accelerated, psycho-emotional stress reduced without causing side effects.
25	Bilyk N.M., 2005 [80]	40 pregnant females	20	In patients receiving combination therapy, the number of term deliveries was twice as high, and the number of stillbirths and spontaneous abortions was half as low compared to the group of patients receiving only conservative therapy.

26	Lisovyi V.N., Yakovlieva E.V., 2006 [27]	54 adults	30	The use of the medicinal product PROTEFLAZID® in patients with genital herpes allows for a significant increase in the effectiveness of the treatment by 15–20%. In addition, the positive effect is more prolonged. The general condition of the patients is significantly improved, immunological indicators are normalized, and the clinical manifestations of herpetic prostatitis are compensated.
27	Gerasimov S.V. et al., 2006 [63]	38 children	17	A faster dynamics of disappearance of aphthous elements (on average on the 5th day of therapy), normalization of temperature, and disappearance of salivation and pain syndrome were noted. The medicinal product PROTEFLAZID® reduced recurrences of the disease: at the end of the day of treatment in the control group, the frequency of repeated visits for gingivostomatitis was 3/10, while in the group of those who took PROTEFLAZID®, no child had recurrences – 0/10 ($p=0.077$).
28	Gopchiuk O.M., 2006 [23]	70 adults	35	In women who received the proposed therapy, a significant increase in the number of CD4+ lymphocytes and NK cells to normal values, an increase in the quantity of IFN- γ and IFN- α , more pronounced positive indicators in the immunogram of women, normalization of the menstrual cycle, absence of recurrences of genital herpes and normalization of vaginal microbiocenosis were observed.
29	Zapolskyi M.E., 2006 [25]	30 adults	30	Positive response in all patients already on the 4th–5th day of treatment, and complete resolution – on the 5th–6th day in 70% of patients were observed. High efficacy in the treatment of genital herpes has been confirmed.
30	Panasiuk O.L., 2006 [69]	236 adults	159	A regression of somato-neurological symptoms was noted on the 8th $\pm$ 2.6th day of treatment; seroconversion of specific antiviral antibodies on the 2nd $\pm$ 0.8th month of treatment; an increase in the number of NK cells by 17%, the functional activity of mononuclear cells by 20%, neutrophils by 10%, and a 1.5-fold decrease in the severity of neuroautoimmune reactions were observed. The drug allows to reduce the frequency of repeated clinical and virological relapses (virus "+") by 1.9 times, and clinical (virus "-") relapses – by 1.3 times.
31	Nagorna V.F., Nikolaeva S.V., 2006 [44]	60 adults	30	PROTEFLAZID® in patients with recurrent miscarriage on the background of HVI contributes to the reduction of relapses and the risk of perinatal losses. The duration of relapse was reduced in most patients by 1.6–2 times, the frequency of relapses – to 1–2 cases in 8 months.

32	Aizatulov R.F., 2006 [29]	30 adults	30	Complex therapy of mixed genitourinary tract infections using the medicinal product PROTEFLAZID® is effective, it does not cause side effects and is well tolerated by patients. Positive clinical dynamics were observed already on the 3rd–5th day of treatment; on the 6th–8th day, discharge stopped, and elimination of the pathogen was observed in most patients.
33	Nagorna V.F., Nikolaeva S.V., 2007 [45]	80 pregnant females	40	PROTEFLAZID® for pre-pregnancy preparation and correction during pregnancy in patients with a history of chronic acute respiratory viral infections and abortions contributes to the correction of immune system function and a decrease in the level of antigenic load.
34	Nagorna V.F., Vinogradov K.V., 2007 [46]	27 children	27	After treatment with the medicinal product PROTEFLAZID® for 3 and 12 months, 26.0±9.1% of children who had acute and/or exacerbation of chronic GI had no laboratory markers of process activity, and 73.9±7.0% of patients with latent course showed a significant decrease in specific IgG titers. The study of the follow-up showed a significant ($p<0.05$) reduction in the frequency of acute respiratory viral infections during the 1st year in 47.8±10.4% of the examined, acute bronchitis in 34.7±9.9%, as well as a significant reduction in the frequency and severity of complications of respiratory tract infections in 39.1% of patients who had undergone ARVI.
35	Nikolaeva S.V., 2007 [47]	140 pregnant females	100	Correction of immunological disorders and improvement of the function of the fetoplacental complex were noted. Reduction of recurrences of herpes infection by 70%, the frequency of threatened abortion by 55%, placental dysfunction by 35%, premature birth by 29.5%, and total perinatal losses by 35% were observed.
36	Sydorenko E.V., Dryianska V.E., 2007 [71]	40 adults	25	A significant decrease in pro-inflammatory cytokines was noted, which indicated a more active suppression of inflammation. The lipid peroxidation-antioxidant system indicators in the blood normalized, which was considered as a potentiated effect of the drug on antibacterial therapy.
37	Shymanska I.G., 2007 [28]	32 adults	32	As a result of the treatment, 15 patients (46.9%) had no episodes of herpes recurrences during therapy, 9 (28.1%) noted a reduction in the duration and activity of clinical manifestations, and in 28 patients (87.5%) after treatment, genital herpes was not detected by PCR, and the virus was not identified in 7 patients with asymptomatic genital herpes.

38	Gerasimova T.V., Hopchiuk O.M., 2007 [24]	150 adults	150	The clinical effectiveness of the proposed treatment regimen was 82.8%. During the treatment period, no exacerbations of the inflammatory process were observed, and the biocenosis normalized, in 64% of women, PCR for HSV was negative; the frequency of recurrences of herpetic infection decreased by 2-3 times.
39	Gley A.I., 2008 [81]	258 adults	112	PROTEFLAZID®, as monotherapy, according to the developed regimen, significantly reduces the duration of viremia for 2 months, compared with patients who did not receive antiviral therapy.
40	Sundukov A.V. et al., 2008 [6]	75 adults	75	PROTEFLAZID® reduces the number of relapses by 8.2–8.4 times and increases the interrelapse period by more than 2.5 times. In the treatment of herpes zoster, the maximum therapeutic efficacy was noted in combination with acyclovir. In the treatment of herpes simplex, the efficacy of the medicinal product PROTEFLAZID® is comparable to acyclovir. In the treatment of infectious mononucleosis, the efficacy of the drug was also noted, which was manifested in positive clinical dynamics and stabilization of hematological indicators.
41	Flax G.A., 2008 [30]	55 adults	35	A reduction in the frequency of exacerbations and normalization of laboratory parameters (disappearance of the virus in PCR tests, decrease and/or disappearance of antibody titer by ELISA during the acute period of GH manifestation and, most importantly, during the period of remission) have been achieved.
42	Chopiak V.V., Potiomkina G.O., 2008 [70]	25 adults	25	PROTEFLAZID® contributes to a significant improvement in the general well-being of the majority (80%) of patients, regression of the main symptoms, has an immunoregulatory effect, promotes an increase in the mass of the killer's subpopulation of lymphocytes through T-cytotoxic lymphocytes, the functional ability of NK cells to synthesize interferons, reduces the suppressive activity of regulatory CD4+/CD25+- lymphocytes, and increases the phagocytic activity of neutrophils, thereby activating acquired and inherent antiviral control.
43	Popova T.V., 2008 [31]	128 adults	98	It was found that the overall effectiveness of the repeated course of therapy was 93.8%, with most patients experiencing a stable regression of inflammatory symptoms and normalization of vaginal microflora.

44	Dolgov G.V., Abashyn V.G., 2009 [48]	62 adults	34	After the end of treatment of patients of the main group, the most favorable adaptive reactions, compared with the initial level, significantly increased by 60%. The increase in natural resistance of patients of the main group is evidenced by the analysis of changes in the indicators of the neutrophil granulocyte system and after complex treatment with the medicinal product PROTEFLAZID®. It has been shown that the administration of the drug significantly and reliably increases the bactericidal activity of blood serum and phagocytosis.
45	Isakov V.A., Yermolenko D.K., 2009 [32]	25 adults	25	The frequency of relapses decreased by 7.5 times and relapses were clinically milder, 32% of patients had no relapses during the year. The average interrelapse period increased by 2.8 times. Positive dynamics were also noted in immunological indicators. After a course of treatment in 23 patients (92%), herpes viruses were not isolated for 6 months by the DIF method.
46	Rykov S.O., Znamenska M.A., 1020 [66]	134 adults	50	PROTEFLAZID® contributed to a faster improvement in clinical ophthalmological indicators in most patients. In the dynamics of treatment, a faster decrease in the intensity of corneal syndrome, discharge in the conjunctival cavity, corneal edema, and inflammatory infiltration was observed. The use of the drug made it possible to reduce the duration of treatment (on average by 4-5 days) and reduce the frequency of relapses by 36%.
47	Sudorenko E.V., 2010 [73]	60 adults	25	PROTEFLAZID® in complex therapy led to more pronounced clinical and microbiological effects, as well as to a decrease in the production of pro-inflammatory IL-8, prosclerotic TGF-β, and the level of antibodies to vascular endothelium.
48	Kamilov Kh.M. et al., 2011 [67]	38 adults	38	Rapid regression of symptoms and restoration of visual acuity were noted (88.9% of cases). Lysis of precipitates took place within 1–1.5 months. The recurrence rate decreased to 5%.
49	Azimova E.I. et al., 2011 [50]	60 pregnant	30	Complex therapy with the inclusion of the medicinal product PROTEFLAZID® prevents the development of immunodeficiency, and improves placental function, which makes it possible to reduce obstetric and perinatal complications in women with recurrent herpetic infection.
50	Romaniuk M.G. et al., 2011 [35]	580 adults	312	On the background of therapy, positive kinetic dynamics, and epithelialization were observed on average on the 6th day of therapy (in the reference group – on the 8th day). The average number of relapses in patients decreased from 6 times per year to 3.

51	Turlibekova S.S., 2011 [59]	48 children	23	Children on day 12 showed positive dynamics of all clinical symptoms with complete elimination of the virus. 1 year after treatment, the level of SLPI in the blood of patients in the observation group significantly decreased and did not differ from the norm ($1,260 \pm 101$, compared to $1,122 \pm 76$ pg/ml, in contrast to patients in the control group with higher indicators ($1,433 \pm 66$ pg/ml).
52	Shamsiev F.M. et al., 2011 [60]	169 children	169	The use of a new treatment regimen of PROTEFLAZID® causes a longer remission and thus demonstrates not only a more pronounced therapeutic but also a stable anti-relapse effect. It has been proven that the dual (immunocorrective and antiviral) effect of the drug is enhanced when used according to the new regimen.
53	Baiev A.I. et al., 2011 [34]	345 adults	80	It has been established that PROTEFLAZID® in the complex treatment of mixed STIs is an effective and safe remedy, which was confirmed by clinical improvement, normalization of immunological and biochemical indicators, and the absence of side effects and adverse reactions.
54	Biletska G.A. et al., 2011 [61]	75 children	30	It has been established that PROTEFLAZID® in the complex therapy of children with IM reduces the duration of manifestations of the disease and the duration of children's hospital stay, and decreases the possibility of recurrences.
55	Zapolskyi M.E., 2012 [26]	88 adults	32	Against the background of therapy, epithelialization of erosions and bullous elements in 96.9% of patients of the main group was completed by the 9th–10th day of treatment. Repeated relapses of MEE during the year were noted in 12.5% of patients. PROTEFLAZID® in the treatment of GMEE has an immunocorrective, antioxidant, and detoxification effect, which is extremely important in herpes-induced autoimmune processes.
56	Benyuk V.O. et al., 2012 [49]	90 pregnant females	30	Positive dynamics in immunological indicators were noted, and obstetric and perinatal pathology was registered 50% less often. The frequency of early pregnancy termination decreased from 73.3% to 36.7%, fetoplacental insufficiency – from 46.7 to 23.3%; premature births – from 16.7% to 12.0%; premature rupture of amniotic membranes – from 46.7 to 20%; development of labor abnormalities – from 26.7 to 10%. The use of the medicinal product made it possible to halve the frequency of pathology in newborns: asphyxia from 43.3% to 23.3%; manifestations of intraamniotic infection from 23.3% to 10%, and development of generalized infection from 11.3% to 0%.

57	Ryzhko P.P., Roshcheniuk L.V., 2012 [35]	110 adults	110	PROTEFLAZID®, as monotherapy, reduced the duration of disease relapses on average from 7.7 ± 0.1 to 4.8 ± 0.08 days, 85 (77.3%) patients demonstrated a decrease in disease severity and relapse frequency by more than 2 times.
58	Klymenko P.M. et al., 2012 [36]	40 adults	30	On the background of therapy, positive clinical dynamics were noted (regression of rashes, reduction of itching, burning) on average by day 4, a 1.5-fold reduction in the duration of relapse, and a decrease in the frequency of relapses.
59	Rak L.M., Yuzko O.M., 2013 [37]	64 adults	32	On the background of therapy, more positive dynamics of regression of clinical manifestations, a stable therapeutic response (after a month of observation), and a decrease in the frequency of relapses were observed.
60	Reznichenko N.A., 2013 [82]	200 pregnant females	200	Against the background of therapy, the clinical manifestations of vulvovaginitis regressed in most patients, the vaginal microflora normalized, and the frequency of registration of diagnostically significant pathogens decreased (by 1.77 times). In immunological indicators, a positive correction was observed in systemic and local immunity, and the level of pro-inflammatory cytokines decreased. 73% of women gave birth to healthy children.
61	Borysova T.P., Tolchennikova E.N., 2013 [73]	54 children	54	During therapy, most patients experienced a reduction in signs of viral activity, a decrease in the frequency of hematuria, an improvement in kidney function, correction of interferon status, a 2.7-fold increase in the level of α -IFN, a 3.6-fold increase in the level of γ -IFN, and a decrease in the level of pro-inflammatory cytokines.
62	Znamenska T.K. et al., 2013 [40]	110 children	40	On the background of therapy, the time spent by patients in intensive care and on mechanical ventilation was reduced, jaundice regressed more quickly, neurosonographic changes (ventriculodilation) were less pronounced, and intracerebral hemodynamics was better.
63	Kolesnik M.O. et al., 2014 [74]	83 adults	20	PROTEFLAZID® contributes to the normalization of cytokine levels in the blood and urine, which indicates the cessation of inflammatory reactions. Faster eradication of pathogens (herpes simplex virus and ureaplasma) was observed, which was manifested by a decrease in the titers of specific IgG antibodies.

64	Kornatskaya A.G., 2015 [38]	70 adults	70	An increase in the level of local immunity indicators was noted. In particular, the level of secretory IgA increased by the 10th day of treatment, remaining significantly high during the 8-week observation period (from 984.32 to 2,496.19 $\mu\text{g/l}$); the level of lysozyme increased by the 10th day of treatment, remaining significantly high during the 8-week observation period (30.06 to 51.67 $\mu\text{g/l}$); the level of C_3-component of complement increased by the 10th day of treatment and returned to the baseline level by the end of the 8-week observation period (from 17.99 $\mu\text{g/g}$ protein at screening to 37.47 $\mu\text{g/g}$ protein on the 10th day and 20.37 $\mu\text{g/g}$ protein on the 8th week). In both groups of subjects, there was a significant decrease in the viral load of HSV DNA compared to baseline. After the completion of the 10-day course of treatment, as well as after the completion of the 2- and 8-week observation periods, HSV DNA was not detected in swabs-scrapes from the epithelium of the vaginal/cervical mucosa in any of the cases. In all subjects, there was a significant decrease in the level of HSV markers (IgG, IgM) compared to baseline after the completion of the 10-day course of treatment, and the 2- and 8-week observation periods.
65	Benyuk V.O., 2015 [39]	70 adults	70	Evidence of the effectiveness of the therapy was a significant increase in local immunity indicators. In particular, the level of secretory IgA and the level of lysozyme increased by the 14th day of treatment, remaining significantly high throughout the entire observation period; the level of the C_3-component of complement increased by the 14th day of treatment and returned to baseline by the end of the 4-week observation period (24.7 $\mu\text{g/g}$ protein at screening; 33.0 $\mu\text{g/g}$ protein on the 14th day; 24.5 $\mu\text{g/g}$ protein by the end of the 4-week observation). A significant decrease in the viral load of HSV DNA was noted compared to baseline. After completion of the 14-day treatment course, as well as after completion of the 4-week observation period, HSV DNA in vaginal/cervical mucosal epithelial scrapings was not detected in all cases. In all subjects, a significant decrease in the frequency of detection of chlamydial DNA occurred compared to baseline.

Conclusions

A systematic review of scientific sources describing the results of preclinical studies indicates that the active substance of the medicinal product PROTEFLAZID® has a direct antiviral effect on DNA contained in herpes viruses. PROTEFLAZID® has a polypharmacological effect: it blocks the synthesis of virus-specific enzymes – DNA polymerase and thymidine kinase in herpes-infected cells, induces the synthesis of endogenous γ - and α -interferons, has antioxidant activity, and exhibits apoptosis-modulating action, enhancing the direct antiviral effect by mediated antiviral activity.

A systematic review of literary sources on the results of many years of clinical use of the medicinal product PROTEFLAZID® (drops) confirmed the wide spectrum of antiviral action against herpes viruses, which in turn confirms the reliability of the results of the preclinical stage of research and proves the effectiveness of the drug in the treatment of diseases caused by herpes viruses in clinical conditions.

A detailed analysis of the results of post-registration clinical studies demonstrates the wide use of the medicinal product PROTEFLAZID® (drops) in children and adults (including pregnant women) in the clinical practice of infectious diseases, pediatrics, ophthalmology, dentistry, gynecology, neonatology, urology, and neurology for the treatment of diseases caused by HV. Long-term results of observations indicate a decrease in the frequency of relapses of HVI after the use of the drug PROTEFLAZID (drops). Based on this scientific evidence, it can be stated that the medicinal product PROTEFLAZID® (drops) is an etiopathogenetically justified drug of choice in the therapy of various clinical forms of HVI in both children and adults in the acute period of the disease and in the period of convalescence.

High clinical efficacy and safety have been confirmed by the results of the use of the medicinal product PROTEFLAZID® (drops) in the treatment of more than 3,200 patients (adults, children, pregnant women) in more than 65 clinical studies with the same focus of the results of the treatment. The drug is well tolerated by patients, without side effects.

The results of preclinical and clinical studies indicate that the medicinal product PROTEFLAZID® (drops) contributes to achieving a stable therapeutic effect, eradication of herpes viruses, and reducing the frequency of relapses of genital herpes.

Given the high efficacy and safety of the medicinal product PROTEFLAZID® (drops) in the treatment of genital herpes, its use is justified and implemented in methodological recommendations and information sheets in Ukraine and abroad.

References:

1. Возіанова Ж. І. Інфекційні та паразитарні хвороби. К.: Здоров'я, 2000. – С. 157.
2. Ісаков В. А. та ін. Герпес: патогенез та лабораторна діагностика: Керівництво для лікарів. С-Пб, 1999. – С. 192.
3. Кубанова А. А., Зудін А. Б. Герпетична інфекція: особливості перебігу, діагностика, проблеми лікарської резистентності. Вісник дерматології та венерології. 2000 (3). – С. 10.
4. Атаманюк В. П. та ін. Новий антигерпетичний препарат Протефлазід. Клінічні проблеми боротьби з інфекційними хворобами Одеса, 2002. – С. 263.
5. ПРОТЕФЛАЗІД: Інформаційні матеріали за властивостями та методиками застосування. Київ, 2002. – 69 с.

6. Скриньок А. В. та ін. Звіт про результати клінічних випробувань препарату Протефлазід у терапії герпесвірусних інфекцій. Москва. 2008. – 17 с.
7. Рибалко С. Л. Проведення додаткових доклінічних випробувань препарату Протефлазід (Звіт). Київ, 2002.
8. Дерябін П. Г. Експериментальне вивчення противірусної ефективності діючої речовини препарату Протефлазід на клітинних моделях ВПГ-1 та ЦМВ інфекцій людини (Звіт). Москва, 2013 року.
9. Рибалко С. Л. Аналіз антивірусних властивостей діючих речовин Протефлазиду та екстраполяція діючих *in vivo* доз на людину (Звіт). Київ, 2011.
10. Рибалко С. Л. Проведення додаткових доклінічних випробувань нових форм препарату Протефлазід (Неофлазід). (Вивчення специфічної дії сиропної форми Протефлазиду на віруси Епштейна-Барр та ВІЛ-інфекції) (Звіт). Київ, 2003.
11. Рибалко С. Л. Вивчення механізмів дії біологічно активних речовин лікувальної субстанції Протефлазід (Звіт). Київ, 2010.
12. Старосила Д. Б. Властивості нових сполук з рослинних флавоноїдів та механізми їх антивірусної дії. Київ, 2014. – 24 с.
13. Завелевич М. П. та співавтор. Інтерферогенна та апоптозмодуюча активність препарату Протефлазід. Вісник Вінницького ДУ, 2002(6). – С. 281.
14. Гриневич О. Й. та співавтор. ПРОТЕФЛАЗІД®: специфічна активність при Епштейна-Барр-вірусній інфекції в умовах доклінічного вивчення; ефективність та безпека в клініці (систематичний огляд). Japan. Educ. and Scient. Review, 2015 (9). – С. 113.
15. Венцківський Б. М. Відкрите дослідження з вивчення переносимості та попередньої оцінки ефективності препарату Протефлазід виробництва ТОВ «НБК «Екофарм» у лікуванні первинної та рецидивуючої інфекції, зумовленої *Herpes genitalis* (Звіт). Київ, 2000. – 21 с.
16. Вовк І. Б. та ін. Використання флавоноїдів у комплексному лікуванні жінок із запальними захворюваннями геніталій вірусно-бактеріальної етіології. Здор. жінок, 2002 (4). – С. 43.
17. Андрієць О. О. та ін. Особливості перебігу вульвовагінітів вірусної етіології у дівчат препубертатного та пубертатного віку. Буковинський медичний вісник, 2004 (1). – С. 15.
18. Андрієць О. О. Комплексне лікування запальних захворювань зовнішніх статевих органів та піхви у дівчаток та підлітків з використанням Протефлазиду. Репродуктивне здоров'я жінок, 2005 (2). – С. 168.
19. Кишакевич І.Т. Удосконалення лікування фонових захворювань шийки матки у жінок з папіломавірусною та герпетичною інфекціями. Львів. 2004. – С. 25.
20. Ромашенко О. В. та ін. Клініко-мікробіологічна, імунологічна оцінка ефективності використання Протефлазиду в комплексній терапії запальних захворювань геніталій у жінок Репродуктивне здоров'я жінок, 2005 (1). – С. 89.
21. Грінкевич Т. М. Ефективність лікування генітального герпесу вітчизняним препаратом Протефлазід. Вісник СумДУ, 2003 (7). – С. 131.
22. Грінкевич Т. М. Сучасні методи лікування генітального герпесу у жінок репродуктивного віку. Київ, 2005. – С. 23.
23. Гопчук О. М. Корекція порушень менструального циклу у жінок з герпетичною інфекцією. Репродуктивне здоров'я жінок, 2006 (1). – С. 187.
24. Герасимова Т. В., Гопчук О. М. Застосування Протефлазиду у комплексній терапії порушень менструального циклу, спричинених вірусною інфекцією. Репродуктивне здоров'я жінок, 2007 (1). – С. 76.

25. Запольський М. Е. Сучасні методи на герпетичну інфекцію. Здор. жінок, 2006 (1). – С. 104.
26. Запольський М. Е. Багатоформна ексудативна еритема, асоційована з герпесвірусом. Епідеміологія та патогенетично обґрунтована терапія. Клін. імунол. Алерг. Інфект, 2012 (8). – С. 1.
27. Лісова В. М., Яковлева Є. В. Препарат Протефлазід у терапії хворих генітальним герпесом. Здор. чоловік., 2006 (1). – С. 138.
28. Шиманська І. Г. Терапія рецидивуючих та малосимптомних форм генітального герпесу. Рецепт, 2007 (6). – С. 97.
29. Айзятулов Р. Ф. Протефлазід у комплексному лікуванні змішаної сечостатевої інфекції у чоловіків. Здор. чоловік., 2006 (3). – С. 133.
30. Флакс Г. А. Нові технології лікування герпесвірусної інфекції із застосуванням препарату Протефлазід. М, 2008. – С. 5.
31. Попова Т. В. Диференційний підхід до комплексного лікування та профілактики запальних захворювань шийки матки у ВІЛ-інфікованих жінок. Одеса, 2008. – С. 21.
32. Ісаков В. А., Єрмоленко Д. К. Ефективність препарату Протефлазід у терапії генітального герпесу (Звіт). С-Пб, 2009. – С. 12.
33. Романюк М. Г. та ін. Використання Протефлазиду при лікуванні генітального герпесу серед чоловіків. Здор. чоловік., 2011 (1). – С. 180.
34. Басв А. І. та ін. Обґрунтування застосування Протефлазиду при мікст-ПСПШ (зі спілкування 1–4). Зап. дерматол. та венерол., 2011 (3–4). – С. 25.
35. Рижко П. П., Рощенко Л. В. Лікування герпесвірусної інфекції із застосуванням препарату Протефлазід у дерматологічній практиці. Клін. імунол. Алерг. Інфект, 2012 (1) – С. 1.
36. Клименко П. М. та ін. Застосування препарату Протефлазід для лікування урогенітального герпесу. Здор. чоловік., 2012 (4). – С. 82.
37. Рак Л. М., Юзько О. М. Застосування індукторів ендогенних інтерферонів у комплексному лікуванні запальних захворювань придатків матки. Здор. жінок, 2013 (7). – С. 138.
38. Корнацька А. Г. Порівняльна оцінка ефективності та переносимості препарату ПРОТЕФЛАЗІД® (супозиторії) та препарату ПРОТЕФЛАЗІД® (краплі) у пацієнток із загостренням герпетичної інфекції (2 фаза). Київ, 2015.
39. Бенюк В. А. Порівняльна оцінка ефективності та переносимості препарату ПРОТЕФЛАЗІД® (супозиторії) та препарату ПРОТЕФЛАЗІД® (краплі) у пацієнток з урогенітальною вірусно-бактеріальною інфекцією (Звіт). Київ, 2015 року.
40. Знам'янська Т. К. та ін. Нові підходи до лікування внутрішньоутробної інфекції у новонароджених від матерів з бактеріально-вірусними мікст-інфекціями Неонатолог., хірур. та перинат. Мед., 2013 (2). – С. 101.
41. Усачова О. В. Ефективність препарату ПРОТЕФЛАЗІД® при лікуванні дітей першого року життя з внутрішньоутробною цитомегаловірусною інфекцією. Репродуктивне здоров'я жінок, 2005 (2). – С. 165.
42. Вдовіченко Ю. П. та ін. Застосування Протефлазиду для профілактики акушерських та перинатальних ускладнень у жінок з герпетичною інфекцією. Репродуктивне здоров'я жінок, 2003 (1). – С. 38.
43. Сімрок В. В., Гордієнко О. В. Ефективність Протефлазиду у профілактиці вірусних інфекцій у вагітних. Проблеми екол. та мед. генетики та клін. імунол. Київ, 2003. – С. 347.
44. Нагорна В. Ф., Ніколаєва С. В. Оцінка ефективності препарату Протефлазід у схемах супресивної терапії герпетичної інфекції у пацієнток зі звичним невиношуванням вагітності. Репродуктивне здоров'я жінок, 2006 (1). – С. 88.

45. Нагорна В. Ф., Николаєва С. В. Механізм імуноспрямованої дії Протефлазиду під час вагітності у пацієнток з невиношуванням вагітності герпесвірусної етіології. Здор. жінок, 2007 (1). – С. 111.
46. Нагорна Н. Ф., Виноградов К. В. Ефективність застосування препарату ПРОТЕФЛАЗІД® при лікуванні герпесвірусних інфекцій у дітей із вродженими вадами серця. Перинатологія та педіатрія, 2007 (1). – С. 76.
47. Николаєва С. В. Предгравідарна підготовка та ведення вагітності у пацієнток з невиношуванням вагітності на тлі герпесвірусної інфекції Одеса, 2007. – С. 25.
48. Долгов Г. В., Абашин В. Г. Досвід застосування Протефлазиду у сімейних пар зі звичною втратою вагітності, спричиненою вірусно-бактеріальною інфекцією. Поліклініка, 2009 (4). – С. 96.
49. Бенюк В. О. та ін. Периконцепційна профілактика та лікування герпесвірусної інфекції у вагітних з метаболічним синдромом. Здор. жінок, 2012 (9). – С. 116.
50. Азімова Е. І. та ін. До питання профілактики рецидиву генітального герпесу у вагітних з антенатальною загибеллю плода в анамнезі Здор. жінок 2011 (2). – С. 238.
51. Резніченко Ю. Г. та ін. Лікування цитомегаловірусної інфекції у дітей із гломерулонефритом. Одеса, 2007.
52. Крючко Т. А. та співавт. Досвід застосування Протефлазиду у комплексній терапії нейроінфекцій у дітей. Імунологія та алергологія, 2002 (3). – С. 60.
53. Єршова І. Б. та ін. Клініко-імунологічне обґрунтування застосування Лаферону та Протефлазиду при лікуванні серозних менінгітів у дітей. Проблеми екологічної та медичної генетики та клінічної імунології. Київ, 2003. – С. 83.
54. Овчаренко Л. С. та ін. Нові шляхи санації внутрішньоклітинної інфекції в педіатрії. Совр. педіатр., 2004 (4). – С. 82.
55. Овчаренко Л. С. та ін. Лікування герпетичної та цитомегаловірусної інфекції у дітей із вторинним імунodefіцитом. Перинатологія та педіатрія, 2006 (3). – С. 60.
56. Усачова О. В. та ін. Досвід застосування препарату ПРОТЕФЛАЗІД® при інфекційному мононуклеозі у дітей. Репродуктивне здоров'я, 2005 (4). – С. 192.
57. Чернишова О. Є. Лікування та реабілітація дітей з різним перебігом герпесвірусної інфекції. Внутрішньоклітинні інфекції та стан здоров'я дітей у XXI столітті, 2005 – С. 9.
58. Чернишова О. Є. та ін. Характер змін здоров'я дітей раннього віку, стану їх імунного та цитокінового статусу на тлі різного перебігу інфекції, спричиненої вірусом Епштейна-Барр. Лікарська практика, 2007 (1). – С. 24.
59. Турлібекова С. С. ПРОТЕФЛАЗІД® у комплексній терапії вродженою цитомегаловірусної інфекції. Валеологія, 2011 (2). – С. 164.
60. Шамсієв Ф. М. та ін. Стан імунного статусу та ефективність Протефлазиду в комплексній терапії дітей з бронхолегеневою патологією, асоційованою з TORCH-інфекцією. Сучасна педіатр., 2011 (2). – С. 39.
61. Білецька Г. А. та ін. Можливі шляхи корекції терапії інфекційного мононуклеозу у дітей. Труднощі діагностики та терапії інфекційних хвороб, Суми, 2011.
62. Яковець В. В., Беліченко Ю. М. Використання протівірусного препарату ПРОТЕФЛАЗІД® у лікуванні вірус-герпетичних захворювань слизової оболонки порожнини рота. Інформаційний листок. № 5. – 2004.
63. Герасимов С. В. та ін. Ефективність Протефлазиду при первинному герпетичному гінгівостоматиті у дітей раннього віку. Репродуктивне здоров'я жінок, 2006 (2). – С. 142.
64. Матяш В. І. та ін. Етіопатогенетична терапія важких форм герпетичної інфекції. Сучасна терапія хворих на інфекційну та паразитарну патологію. Харків, 2002.

65. Петруня А. М., Воротніков С. В. Ефективність Протефлазиду в комплексній терапії хворих з рецидивуючим герпетичним кератитом. Проблеми екологічної та медичної генетики та клінічної імунології. Київ, 2003. – С. 309.
66. Ріков С. О., Знаменська М. А. Сучасні погляди на етіологію та лікування герпетичних кератитів. Новини медицини та фармації. Офтальмологія, 2010. – С. 324.
67. Камілов Х. М. та ін. ПРОТЕФЛАЗИД® у комплексному лікуванні герпетичних захворювань очей. Збірник наукових праць НВК з офтальмохірургії з міжнародною участю. Уфа, 2011 р.
68. Гошко О. Л. та ін. Вивчення інтерференогенної та імуномодуючої активності Протефлазиду у хворих з герпесвірусним ураженням нервової системи. Лабораторна діагностика, 2005 (4). – С. 30.
69. Панасюк О. Л. Етіопатогенетична терапія герпесвірусної інфекції з використанням Протефлазиду та ультрафіолетового опромінення крові. Київ, 2006. – С.18.
70. Чоп'як В. В. та ін. Ефективність монотерапії препаратом ПРОТЕФЛАЗИД® при лікуванні хворих на хронічну EBV-вірусну інфекцію в стадії реактивації. Імунологія та алергологія, 2008 (1). – С. 3.
71. Сидоренко Є. В. та ін. Вплив Протефлазиду на продукцію цитокінів та систему ПОЛ-АОЗ у хворих на хронічний пієлонефрит. Сімейна медицина, 2007. – С. 34.
72. Сидоренко Є. В. Фактори міжклітинної кооперації імунної системи та можливості імунокорекції у хворих на хронічний пієлонефрит. Київ, 2010. – С. 24.
73. Борисова Т. П., Толченнікова Є. М. Нові підходи до терапії дітей з гематуричною формою хронічного гломерулонефриту та супутньої хронічної ЕБВІ. Пробл. пін. педіатрії та вищої медичної освіти. Донецьк. 2013. – С. 269.
74. Колесник М. О. та ін. Вплив імунокорекції на стан цитокінової ланки і SLPI у хворих на пієлонефрит. Український журнал нефрології та гемодіалізу, 2014 (1). – С. 19.
75. Луцик Б. Д. та ін. Застосування препарату ПРОТЕФЛАЗИД® у лікуванні вірусних ендocerвіцитів, ерозій шийки матки. Медицина залізн. транс. України, 2003 (4). – С. 57.
76. Федотов В. П. та ін. Застосування Протефлазиду в лікуванні хворих на рецидивуючий генітальний герпес. Дерматологія. Косметологія. Сексопатологія, 2003 (2-3). – С. 5.
77. Шведюк С. В. та ін. ПРОТЕФЛАЗИД® у лікуванні урогенітального хламідіозу, що поєднується з генітальним герпесом. Пробл. екол. та мед. генетики та клінічної імунології. Київ, 2003. – С. 376.
78. Нікіфорова Т. О. та ін. Клініко-імунологічна ефективність Протефлазиду у хворих на Епштейна-Барр-вірусну інфекцію. Тернопіль, 2004. – С. 158.
79. Глей А. І. Інфекційний мононуклеоз, етіологічні та клінічні особливості. Київ, 2008.
80. Білик Н. М. Діагностика та лікування ретрохоріальних та ретроплацентарних гематом у жінок з антифосфоліпідним синдромом та вірусною інфекцією. Педіатрія, акуш. та гінек., 2005 (2). – С. 91.
81. Глей А. І. ПРОТЕФЛАЗИД® у лікуванні хворих на інфекційний мононуклеоз, спричинений вірусом Епштейна-Барр. Сучасні інфекції, 2005 (3-4). – С. 121.
82. Резніченко Н. А. Імуномодулятор у лікуванні перинатальних інфекцій. Пит. експериментальної та клінічної медицини, 2013 (17). – С. 3.

Reference: Matyash V. I., Hrynevych O. Y., Panasiuk O. V., Solomakha L. PROTEFLAZID®: specific activity against infections, caused by Herpes virus under the conditions of preclinical study and efficiency/safety of its use in clinical practice (systemic review) // *Yale Review of Education and Science*. – 2015. – No.1. (16). – P. 423–462.

2.2. PROTEFLAZID®: CLINICAL EXPERIENCE IN CHILDREN OF YOUNG AND PRESCHOOL AGE – SYSTEMATIC REVIEW OF POSTMARKETING SURVEILLANCE

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Abstract

Scientific literature data on the experience of use of PROTEFLAZID® (drops) and FLAVOVIR® (syrup) for the treatment of viral diseases in children of the first six years of life are analysed in the article. A systematic review was conducted on the basis of postmarketing comparative clinical trials and long-term follow-up (during the period of 2002 to 2016) that involved about 1500 children (the intent-to-treat population comprised more than 800 of them). The safety and efficacy of the PROTEFLAZID® (drops) and FLAVOVIR® (syrup) usage in children for the treatment of viral infections have been proven.

Key words: PROTEFLAZID®, FLAVOVIR®, herpetic infections, ARVI, treatment, children.

Introduction

Relevance. In clinical practice, the prophylaxis and treatment of viral infections take a special place due to their high prevalence in children as well as in adults. It is well known that 80-90% of population are infected with Herpes simplex viruses type I/II, Epstein-Barr and cytomegalovirus – up to 70%, therefore herpetic pathology is one of the most important problems of modern paediatrics and neonatology [8]. Concerning the acute respiratory viral infections (ARVI), they have a persistently high level in the infectious morbidity pattern in both the whole world and Ukraine. Among children under 17 years of age, its level makes up more than 60,000 cases per 100,000 population (3.3 times higher than in adults, including influenza – 979, exceeding the level in adults by 2.8 times) [5]. In Poland over the last years, respiratory infections account for about 40% of all hospital admissions annually. This index reaches 90% in infancy and up to 70% for the initial four years of life [1]. For children who begin to visit pre-school facilities, repeated (recurrent) respiratory infections are specific characteristic. These particular children constitute a risk group for the development of chronic diseases of the nasopharynx and the bronchial asthma, since the immunity status post ARVI is species- and type-specific, which causes high incidence [1,7,34].

In management of viral infections, the elimination of pathogen and the host immune response have a great influence on the recovery. In the world practice it is considered that

the shortest and most effective management of viral pathology is possible by affecting an aetiological agent. However, the aetiological therapy of such diseases often presents itself as challenging considering the enormous numbers of pathogens and the paucity of selective drugs with proven clinical efficacy and safety. Several product groups have been discovered to date, the action of which is based on the suppression of viral replication. However, their application in paediatric practice is limited due to the high toxicity, since the metabolism of viruses' multiplying is closely related to the metabolism of the host's cells, and the effect on the virus almost inevitably affects the cells of the growing and developing child's organism. In addition, the certain challenges emerge in chronic viral infections management, which are as follows: it is not always possible to achieve a positive result with the help of aetiological therapy in the immunocompromised patients or in case of oxidative stress (OS). Consequently, a complex of treatment and rehabilitation measures should include drugs that normalize the immune system (IS) functioning and suppress the hyperactivity of free radical processes. It is known that aetiological agents destroy or inhibit the activity of the pathogen, while immunotropic therapy directly or indirectly increases the functional activity of phagocytes, enhancing their anti-infectious effect, stimulating the reaction of the cellular and humoral component of IS. OS control increases the degree of cell membranes protection improving the effectiveness of treatment and rehabilitation measures.

The problem solution of viral infection treatment efficiency improvement become possible now through the use in the general medical practice on a wide scale basis of the brand-name drug Proteflazid® (drops) (manufactured by the Ecopharm Research and Production Company, Ukraine), the active ingredient of which is derived from the plants *Deschampsia caespitosa* L. and *Calamagrostis epigeios* L. The major biologically active component of the active ingredient is a complex compound of O- and C-glycosidic flavones: tricine, luteolin and apigenin. For children, Proteflazid® is also recommended in the syrup dosage form, manufactured under the brand name Immunoflazid®.

The medicinal preparation Proteflazid® is characterized by a number of basic pharmacodynamic properties as follows:

- etiotropic action: a) inhibits the virus-specific enzymes synthesis of the RNA- and DNA-viruses (thymidine kinase, DNA- and RNA-polymerases, revertase), which is accompanied by impairment in ability or total block of the viral nucleic acids' replication and, as a result, makes the RNA- and DNA- viruses' reproduction impossible; b) inhibits the neuraminidase activity of influenza viruses, preventing them from entering the host through the respiratory tract mucous lining or the yield of viruses from the infected cells, reducing the risk of bacterial-associated complications (e.g. pneumonia) [3, 4, 7];

- pathogenetic action: a) it has an immunotropic mode of action, namely optimizes the endogenous α - and γ -interferons (IFN) synthesis, restores tissue immunity by increasing the secretory immunoglobulin A (sIgA) level; b) it has an antioxidative mode of action (in pre-clinical studies it has shown that the active ingredient of drug is an effective agent to speed control of the superoxide radicals generation by the normal and transformed cells, the agent at a dose of 5 mg/ml, nulls the superoxide cells generation). The drug reduces the lipid peroxidation activity and enhances the antioxidant defence activity, which results in reduced endotoxemia and increases the resistance of cells and tissue to damaging action of the free-radical oxidation products, the level of which is increased during the most pathological processes, especially in cases of inflammation and infection [1,23].

Polypharmacologic features (direct antiviral, immunotropic and antioxidant modes of action) of Proteflazid® provide both etiologic and pathogenic effects on both the viral infection and pathological processes in the host caused by this infection. The above mentioned properties of the medicinal preparation Proteflazid® are the basis for its wide application in various fields of medicine – prevention and treatment of a number of infectious diseases caused by such viruses as: Herpes viruses types I and II, Epstein-Barr virus, human papilloma virus, hepatitis viruses, influenza virus, cytomegalovirus (CMV) and others, as well as in the complex therapy for viral-bacterial and fungal infections [7]. The activity of drug is equally high in both the first hours of the disease, and the following days [2, 19, 21, 33].

Due to lack of a real opportunity in practical medicine to accurately determine the pathogen type of the infectious process in the first day of illness, physicians rely only upon their own experience in choosing etiologic therapy, in both assessing the symptoms of the disease and using etiologic antiviral agents [14].

Given the etiopathogenic aspects of viral diseases (including influenza and ARVI), the routes and mechanisms of infection, the options for their treatment should have the following basic pharmacodynamic properties: – direct broad spectrum antiviral action (act on RNA- and DNA-viruses) at all stages of the viral infection development; – to suppress neuraminidase activity of influenza viruses; – the superior bioavailability to the upper respiratory tract mucosal lining; – detoxicative and antioxidant characteristics; – to show an immunotropic effect against a background of high-grade security, bioavailability and efficacy [14, 26].

Experimental studies with laboratory animals and cell cultures carried out at the stage of preclinical research have proved that the medicinal preparation Proteflazid® has specific antiviral activity against influenza virus, Herpes simplex, Epstein-Barr virus, hepatitis virus, adeno-, papilloma and CMV, HIV, no adverse and toxic effects, does not create a pharmacological loading on the detoxification and excretion organs; has a mild immune stimulating effect, on account of which it is recommended for paediatric use [4, 11]. However, despite the long-term experience of a line of medicines based on Proteflazid® in medical practice, the possibility of their use in children of early and preschool age raises a number of questions among many paediatricians, primarily regarding its safety and the possible impact on the child's postnatal development. We tried to answer such questions in the present systematic review.

Objective of the study

On the basis of postmarketing comparative clinical trials and follow-ups, to conduct an analysis of scientific and practical data of the evidence base that confirms the efficacy and safety of the medicinal preparation Proteflazid® (drops) and Immunoflazid® (syrup) for the treatment of diseases of viral aetiology in children of the first 6 years of life.

Materials and methods

Scientific publications, clinical trial reports, systematic analysis were under evaluation in the study.

Results and discussion

In a systematic review, the results of studies of Proteflazid® (drops) and Immunoflazid® (syrup) in clinical practice published during the period of 2002 to 2016 were analysed.

A considerable part of the scientific data is devoted to the clinical experience of the medicinal preparation Proteflazid® for opportunistic infections, the development of which occurs conditions of immune deficiency. Herpes simplex viruses types I and II, CMV and chlamydial infections rank high up among them. An infection level increase among women of fertile age with these pathogens leads to the fact that the infectious pathology of foetus and newborn becomes one of the most important issues of modern neonatology. The incidence of this pathology varies from 6% to 53% and tends to increase, while CMV takes the first position among its aetiological factors. In a newborn, along with the acute infection, a long-term persistence of pathogen can be noted with the formation of latent infectious disease, an immunodeficiency state, which, in subsequent years, may become one of the factors of the recurrent infectious morbidity. Ambiguity regarding the necessity and time frame of the toxic enough antiviral chemotherapy stimulates the scientists to search for effective mode therapy of this pathology. Thus, Usachova Ye.V. [35] has showed the effectiveness of Proteflazid® (drops) for the intrauterine CMV infection in infants. Evaluation of the results of the dynamic clinical and laboratory study demonstrated a nulling of the cytolytic syndrome, an achieved positive serological result, which manifested itself in the disappearance of IgM against CMV and a decrease of anti-CMV IgG. Such results are regarded as an evidence of an antigen load reduction, i.e., suppression of viral replication. In children with liquor hypertensive syndrome, according to the neurologist examination results and the control neurosonography data, stabilization of condition and the absence of a further enlargement of the third and lateral ventricles of cerebrum were noted.

The observation results made by Znamenskaya T.K. et al [16] also showed the efficacy of Proteflazid® (drops) in the complex therapy of children with intrauterine infection, which was manifested by a positive dynamics of the clinical symptoms of the disease, a decrease in the duration of stay of newborns in intensive care unit, a decrease in the duration of invasive artificial lung ventilation, leading to reduction of treatment expenses of this category of patients. Neurosonography and Doppler ultrasound of the cerebral haemodynamics confirm the high treatment efficiency of intrauterine infection with Proteflazid® (drops).

To date it is known that among the newborns, the high risk group consists of premature neonates, and among them – small-for-date newborns, who have the most pronounced and persistent immunological defects [12]. Considering the important role of OS reactions in the formation of both compensatory and pathological mechanisms of postnatal adaptation of pre-matures, especially under conditions of hypoxia, the status of indices of pro-oxidant and antioxidant defence systems has been studied. Godovanets Yu.D., Babintseva A.G. [11] showed the effectiveness of the Proteflazid® (drops) in the complex treatment of an acute period of hypoxic-ischemic CNS injury in premature neonates during the early neonatal period based on the antioxidant defence system and some immunological indices. The positive effect of drug on the postnatal adaptation, a significant decrease in the extra-neural manifestation rate of hypoxia was shown. The results of laboratory tests (complete blood cell count, pro-oxidant and antioxidant protection system indices) confirmed the efficacy of Proteflazid®.

Intrauterine infections are often responsible for child's disability due to congenital defects (CD) and background chronic diseases [6]. Nagornaia N.V., Vinogradov K.V. [23] presented the results of the study of the Proteflazid® (drops) effectiveness for various course of herpetic infections in children with congenital heart defects. It was shown that 3 and 12

months later after the treatment, which was conducted taking into account the differentiation according to the infection stage, in a significant proportion of children, who had an acute and / or an exacerbation of the chronic herpetic infections, the lack of activity laboratory markers, a significant decrease in specific IgG titres was revealed in more than 70% of patients with latent disease. The prospective follow-up during the first year after the treatment indicated a significant decrease in the incidence of ARVI, acute bronchitis, a decrease in the frequency and severity of complications of respiratory tract infections in patients who were administered the agent.

The objective of the research of Veselyi S.V. and Kliman- skii R.P. [9] was to study the effect of Immunoflazid® on the state of the IS activity in children with the gastrointestinal tract CD on condition of the different course of infection caused by persistent intracellular pathogens. A significant decrease in activity of proinflammatory cytokines (IL-1, IL-2, IL-6), INF- γ and TNF- α and the increase of anti-inflammatory cytokines (IL-10 and INF- α) activity served as a drug response rate. Immuno-flazid® in the complex therapy, as well as in monotherapy, allowed to eliminate the IS imbalance in a shorter time period, induce the treatment terms reduction, without exerting a negative influence on the child's organism.

It has been proved that Herpes simplex viruses, Epstein- Barr virus, CMV persist in organism for a long-term period and, in case of its reactivity decrease, they may affect the cardiovascular, digestive, nervous, lymphoid, respiratory systems, provoking frequent development of pneumonia. Ovcharenko L.S. et al. [24] showed a beneficial effect of Proteflazid® (drops) on intracellular pathogens (CMV, Chlamydia) for the treatment of ARVI and the prevention of bacterial complications in such children. Dynamics of immunological indicators confirmed the anti-inflammatory effect of Proteflazid® due to the infectious antigen loading decrease. Repeated course of treatment of the resistant forms of disease in 1.5-2 months allowed achieving normalization of laboratory parameters in 100% of cases.

The necessity and effectiveness of Immunoflazid® syrup usage in the complex treatment of community-acquired pneumonia in young children secondary to CW and/or Herpes virus (types I, II) infections was investigated by Zalizyuk A.A. [15]. It was shown that the drug, as part of complex treatment, prevents the lingering disease, reduces hospital stay, restores the endocrine function of thymus, T-cell immunity parameters, and arrests the clinical manifestations of pneumonia along with lack of adverse effects.

Turlibekova S.S. [29] also showed that effective treatment of CMV infection can be provided only by applying in the complex therapy – etiotropic and pathogenetic. This approach grounds on a lifelong virus persistence in a host-organism. The authors have proved the clinical laboratory effectiveness of combined therapy that included Proteflazid® (drops) along with interferons.

Grigorchuk N.V. et al. [13] managed to arrest the acute herpetic infection in 58 children, and to prevent the formation of its clinically manifested relapses. All examined children had a twofold decrease in the number and duration of recurrent respiratory diseases and the frequency of their bacterial-associated complications.

A primary herpetic stomatitis is a widespread manifestation of primary Herpes simplex virus infection in children. Gerasimov S. V. et al. [10] showed that Proteflazid® (drops) taken in therapeutic dose reduces the duration of primary acute herpetic gingivostomatitis by 2-3 days, reduces the frequency of its recurrence in infants and young children, and is comparable in effectiveness to acyclovir.

Shamsiev F.M. et al. [27, 32] in their study confirmed the significant pathogenetic role of immune disorders in the formation of acute pneumonia associated with TORCH infection. The complexity of pathogenesis, the cascade of pathological processes and the variety of implementation mechanisms, as well as the deep of immune destruction, point out the necessity of including a drug with an antiviral and immunocorrecting effect in the treatment of pneumonia in such children. The authors proposed a new treatment regimen with a long-term intake of the drug for 5-6 months. Comparative analysis of the main parameters of the immune system showed that Proteflazid® (drops) as part of complex treatment under the new scheme promotes the faster improvement of clinical and immunological dynamics, increases the activity of immunoregulatory subpopulations of T- lymphocytes, non-specific factors of defence, for recurrent pneumonia in children with herpetic infection.

It is well known that early and preschool children's age (6 months – 6 years) is characterized by increased child's sensitivity to ARVI, which is their ontogenetic feature [7]. As a rule, at onset of disease, it is difficult to determine its viral aetiology. In this regard, the ability of an antiviral agent to be administered to influence both the RNA and the DNA viruses that cause the onset of ARVI acquires particular relevance [26].

In preclinical studies and in clinical practice, it has been found that Proteflazid® (drops) and Immunoflazid® (syrup) inhibit the replication of both RNA- and DNA-viruses via blocking the specific for all viruses enzymes (RNA- and DNA-polymerase, thymidine kinase, revertase), and also inhibit neuraminidase of influenza viruses. These properties of drugs are very important for the implementation of preventive and treatment measures in conditions where it is difficult to determine the aetiology of the disease [14].

The children of the infant orphanage are the most susceptible to ARVI category of the child population. The increased morbidity of this population is associated with multiple deviations in their health status and a violation of the host defences. The effectiveness and safety of the preparation Immunoflazid® for the prevention and treatment of ARVI among children in the orphanage was studied by Yulish Ye.I. et al. [33, 35]. The clinical effectiveness of the drug in the treatment and prevention of ARVI in children of early age is proved. Against the background of the drug usage, the incidence of the severe disease and its complications decreased, and from the third day of treatment a positive clinical dynamics was observed. In addition, the incidence of ARVI decreased 2.3 times that confirms the preventive effect of Immunoflazid®. Yulish Ye.I. et al. [36] recommend Immunoflazid® for use in healthy children and those with recurrent respiratory diseases for the purpose of non-specific prevention of ARVI during the seasonal increase of ARVI.

Zubarenko A.V et al. [17] also confirmed the efficacy of Immunoflazid® in the prevention of infectious diseases in infants on the background of lack of allergic reactions and digestive tract disorders.

In children with recurrent infections, the IS is characterized by the intensity of the response processes and the inadequacy of reserve capacities, so the issues of their effective rehabilitation is a question of great importance. The purpose of research conducted by Zyuzina L.S., Myzgina T.I. [18] was to determine the clinical efficacy of Immunoflazid® in the complex therapy of young children with recurrent respiratory diseases. It was shown that in patients who were treated with the drug, compared with the control group, the disease course was mild. Clinical symptoms (intoxication, fever, bronchial

obstruction) and general condition improved in a shorter period of time. The hospital stay of patients decreased by 1.2 times. In the catamnesis it was determined that the frequency of repeated hospital admission of children with complications of viral infection, who were administered the drug, was 3 times less than in the control group. The authors noted that Immunoflazid® is an effective, easy to use, well tolerated by children and has no adverse effects.

In the study of Tokarchuk N.I. and Starinets L.S. [33] it was shown that the preparation Immunoflazid® in children of early age with ARVI and influenza contributes to the restoration of the functional state of thymus (normalize thymulin level), and the T-cellular component of IS parameters, the integral coefficients of non-specific protective factors. The inclusion of Immunoflazid® in the treatment of ARVI in young children reduces the duration of hospital stay.

Kinash Yu.M. [23] confirmed that the underlying immune deviations in children with recurrent bronchitis relate mainly to thymus-dependent lymphocytes. There was a significant decrease in both the main T-cell pool and subpopulation of T-helpers CD4, as well as a decrease of T-suppressors CD8. Immunoflazid® contributed to normalization of those indicators levels.

Tsimbalista O.L., Garidzhuk L.I. [36] studied the levels of IL-4, IL-6, IL-8, IL-10, and INF- α in 90 young children with complicated community-acquired pneumonia secondary to iron deficiency anaemia (IDA). An increase of cytokines in proportion to the iron deficiency degree was observed in the examined children. After clinical and radiological recovery, the active inflammation in lungs intensified to the extent of the iron level decrease. That was confirmed by an increase of the IL-6, IL-8 and TNF- α levels. The combination of basic therapy of pneumonia with the preparation Immunoflazid® improves the therapeutic effect by reducing the activity of inflammation at all levels of concomitant IDA. It has been shown that Immunoflazid®, due to its anti-inflammatory properties, is effective and safe for use in infants with complicated pneumonia on the background of IDA.

The results of the clinical management analysis confirm the effectiveness of the preparations Proteflazid® (drops) and Immunoflazid® (syrup) for the treatment of both the active viral infections and the latent diseases in children, providing direct antiviral, immunocorrecting and antioxidant actions. Mono- and complex therapy with these drugs, allow the elimination of pathogens within a short time period, normalize the IS imbalance, shorten the time of treatment and intake of concomitant medications; reduce the risk of repeated episodes and complications developing. The listed therapeutic effects give grounds for the wide use of Proteflazid® and Immunoflazid® in children with herpetic infections, ARVI and influenza, as they fully correspond to the etiopathogenetic requirements for the prevention and treatment of these diseases.

It should be emphasized that the essential advantage of these preparations Proteflazid® (drops) and Immunoflazid® (syrup) – over other drugs that are used in mono- and combination therapy of viral and viral-bacterial diseases is that these drugs – analysis have both etiotropic and pathogenetic potential, and in recommended doses they are safe and indicated for treatment in children from birth. The use of these drugs makes it possible to avoid unreasonable polypragmasy and save parents' financial resources.

The table presents the main results of 19 clinical trials in chronological order.

Table 1. Results of clinical trials on the efficacy and safety of the medicinal preparations Protefiazid® (drops) and Immunoflazid® (syrup) in young children during the period of 2002 to 2016

№	Authors, year, / source	Pathology	Number of patients		Main results of drug use in clinical practice
			total	intent-to-treat	
1.	Kryuchko T.A et al., 2002 [22]	Neuroinfection	20	20	GP: 1* of intoxication, improvement of well-being, rapid arresting of meningeal syndrome, N of the CD4/CD8 correlation from 0.7 ± 0.21 to 1.3 ± 0.32 , T of serum IgG from 3.8 ± 0.8 g/L to 8.4 ± 1.8 g/L; IgA from 0.1 ± 0.6 g/L to 3.3 ± 0.05 g/L.
2.	Godovanets Y, Babintseva A, 2005, [11]	Hypoxic-ischemic CNS injury in premature neonates	54	22	GP: stabilization of general health status in 95.5% vs CG: 46.9%, $p < 0.05$. Peristalsis depression – 2.5% vs 43.8%, respectively, $p < 0.05$; hepar enlargement – 9.1% vs 65.6%, respectively, $p < 0.05$. The significant higher activity of plasma catalase (1.7 ± 0.11 U/min. 1 g protein and 1.2 ± 0.08 U/min. 1 g protein, respectively, $p < 0.05$), the ceruloplasmin level (46.8 ± 1.08 U/1 g protein and 25.2 ± 1.24 U/ 1 g protein, respectively, $p < 0.05$) and the level of sulphhydryl-groups of plasma (1.7 ± 0.03 pmol/ 1 g protein and 0.8 ± 0.05 pmol/1 g protein, respectively, $p < 0.05$). GP: 1 in the intensity of oxidative modification of proteins was $70.611.88$ U/1 g protein vs CG: $106.912.79$ U/ 1g protein, ($p < 0.05$) in.
3.	Usachova Ye.V., 2005 [30]	Intrauterine CMV infection	54	23	GP: 1 ALT to normal limits at 814.5 day of treatment. A positive serological effect in 52% cases: the disappearance of anti- CMV IgM and 1 of anti-CMV IgG as an evidence of an antigenic burden reduction as a result of viral replication depression. Stabilization of children with liquor hypertensive syndrome and the absence of a further enlargement of the third and lateral ventricles of cerebrum.

4.	Ovcharenko L.S. et al., 2005 [24]	ARVI against a background of chronic persistence of intracellu- lar pathogens (chlamydia and CMV)	60	30	GP: 1 quantitative parameters of killer cells (CD8, CD 16). Antioxidant action of the drug: I (to normal values) of the tetrazolium test. T IgG and IgA against 1 IgM. After the course of treatment in 2 children with CMV, a high antibody titre of anti-CMV IgG 1:20, IgM 1:100 remained. After the repeated course of therapy: 1 IgG titre to 1:4 and IgM to 1:10.
5.	Gerasimov S.V., 2006 [10]	Primary her- petic gingivo- sto-matitis	34	17	GP: 2-3 days earlier rashes in the oral cavity disappeared, N the body temperature, appetite and salivation disappeared; the total number of doses of non-steroidal anti-inflammatory drugs was significantly less (GP: 12+3 vs CG: 1715, $p=0.001$). Proteflazid® helps reduce the recurrence of disease: CG: 3/10 cases of recurrence, GP: no relapses.
6.	Nagornaia N.V., / Vinogradov K.V., 2007 [23]	Herpetic infec- tions in chil- dren with congenital heart defects	27	27	GP: In 3 months after treatment, there was lack of laboratory markers of herpetic infections. At the same time levels of HSV IgG remained high in 1 child with acute infections. 1 the titre of specific IgG was observed more than 2-fold in 13 children. 1 specific IgG in patients with latent herpetic infections. One year later after the treatment, the children had no signs of herpetic infections activity (EUSA, PCR). The diagnostically insignificant IgG titres were recorded in 10.0% of children with the exacerbation of chronic herpetic infection and in 100.0% with latent herpetic infections. 1 the frequency of respiratory diseases (from 91.315.8 to 43.4+10.4%) and acute bronchitis (from 56.5+10.3 to 21.7+8.6%). 1 the proportion of diseases with mild course (69.519.5%) as compared with the baseline data. 1 more than 2 times the number of complicated forms of respiratory infections, I the frequency of antibacterial drugs administration (52.1110.4%), compared to the data before treatment (82.617.9%).

7.	Grigorchuk N.V., 2008 [13]	Neonates with herpetic infections	100	100	GP: Acute manifestations of infection in 58 children were stabilized; the recurrent course of chronic herpetic infection was transferred to the latent infection. 2-fold 1 the number and duration of recurrent respiratory diseases and the frequency of their bacterial-associated complications.
8.	Sagatova M., Shamsiev F.M., 2009 [27]	Recurrent pneumonia in children with herpetic infections	58	38	GP: T CD3, CD4 and CD8 lymphocytes ($p<0.001$) in 3 months from the initiation of the drug administration. N phagocytic activity and the concentration of IgG and IgM in the blood serum, compared with the baseline data ($p<0.001$).
9.	Yulish Ye.I. et al., 2009 [36]	HRVI (prevention)	80	55	GI: the number of patients with ARVI was 22.2% ($n=6$) of children vs CG; 56.0% ($n=14$). Thus, during the epidemic season, the use of Immunoflazid® can protect against the disease 77.8% of young children. GI: ARVI course was mild, lack of severe forms, the average duration of ARVI was 3.3 ± 0.7 days, besides clinically they had catarrhal manifestations secondary to the low-grade temperature, which were arrested by the end of the fourth day vs CG; the duration of ARVI was 9.711.6 days, which was due to the development of complications (otitis, bronchitis).
10.	Kinash Yu.M. 2010 [19]	Recurrent bronchitis	65	45	GP: in 3 months after treatment, the cellular and humoral components indices of immunity: 1 the number of T-cells due to CD4 and CD8 ($p<0.05$). 2-fold 1 the IL-2, IL-10 values. The dynamics of local immunity indices showed 1 of the lactoferin and sIgA levels.
11.	Turiibekova S.S., 2011 [29]	Intrauterine CMV infection	48	23	GP: the significant positive dynamics of clinical symptoms (I temperature to normal values, arrest of dyspeptic syndrome, improvement of appetite and emotional tone of children).

12.	Shamsiev N.Kh. et al., 2011 [32]	Broncho-pulmonary pathology associated with TORCH infection	169	82	GP: 1 the number of CD3-lymphocytes ($p<0.001$), CG: these values did not reach normal limits. GP: the performed immunocorrection contributed to 1 in the number of CD16-cells. The parameters of humoral immunity component (the number of CD20+ cells, IgA, IgM and IgG levels), as well as the phagocytic activity of leukocytes were close to the control values.
13.	Zalizyuk A.A., 2011 [14]	Community-acquired pneumonia in children secondary to CMV and/or Herpes simplex virus types I, II	50	25	GI: there was a reduction of terms of intoxication syndrome (6.67 ± 0.32 days vs CG: 10.7 ± 0.8 days) ($p<0.05$); GI: 1 the duration of inpatient treatment by 6.6 ± 1.3 days against CG ($p<0.05$). GI: significant changes in the parameters of the immunological status in comparison with CG: N CD4+ T-lymphocytes in comparison with the initial levels. GI: N the thymulin level and prevention thymus mass reduction.
14.	Tokarchuk N.I., Starinets L.S., 2012 [28]	ARVI and influenza	50	25	GI: N CD+ T-lymphocytes (CD3+, CD4+, CD8+, CD16+), immunoregulatory index CD4/CD8 ($p<0.01$), CD4+ T-helpers compared with baseline data ($35.1\pm1.2\%$ vs $42.7\pm1.2\%$, $p<0.05$); t phagocytic protection and 1 the specific immune lymphocyte-monocyte potential in comparison with baseline data ($p<0.05$) GI: N the thymulin level and prevention the thymus mass reduction: 1 by 53.6% the thymulin level ($p<0.05$) vs CG: I by 32.3% the thymulin level in the blood serum during the treatment ($p<0.05$).

15.	Tsimbalista O.L., Garidzhuk L.I., 2013 [31]	Complicated community-acquired pneumonia secondary to iron deficiency anaemia	80	40	GI: I the duration of inpatient treatment ($p<0.05$). I pro-inflammatory cytokines level, i.e. I the therapeutic effect by reducing the activity of inflammation at all degrees of concomitant iron deficiency anaemia.
16.	Znamenskaya T.K. et al., 2013 [16]	Intrauterine infections	80	40	GP: I the duration of the intensive care unit stay of newborns (13.4 ± 0.3 days vs CG: 17.510.5 days), I the artificial lung ventilation duration ($8.410.3$ vs 11.2 ± 0.2, respectively). GP: the rapid disappearance of jaundice: at the end of the first month of postnatal period, its incidence was 7.5% vs CG: 12.5%. At the 3rd week of life, the signs of ventriculodilation (the ventricular index increase and the ventriculometry data) (neurosonography), which indicated a violation of haemocerebrospinal fluid circulation. GP: the above mentioned indicators were lower ($p<0.005$); interventricular asymmetry was less common; the width of the vascular plexuses was significantly less; the frequency of periventricular leukomalacia was lower. More intensive improvement of cerebral haemodynamics (doppler ultrasonography): significantly higher blood flow velocity, decrease of pulse and resistant indices ($p<0.05$).
17.	Zubarenko A.V. et al.	Infectious diseases in infants born from mothers who are TORCH infection carrier	75	39	GI: I the incidence of disease: 17.94% of children vs CG: 83.33%. GI: the disease duration among children becoming ill was 610.7 days vs CG: 8.2510.45 days.

18.	Zyuzina L.S., Myzgina T.I., 2015 [18]	Children with respi- ratory recur- rent diseases (prevention)	78	40	Gl: the disease course was mild, compared with CG, the disappearance of intoxication symptoms, fever, obstructive syndrome and general condition improvement occurred in a shorter period. Gl: I by 1.2 times the hospital stay of patients, com- pared with CG.
19.	Veselyi S.V., Klimanskii R.P., 2016 [9]	Congenital defects of the gastrointestinal tract, asso- ciated with the persistent intracellular pathogens	87	64	Gl: I the specific immunoglobulins level that before treatment exceeded the upper limit of normal by 8-9 times; 1 activity pro- inflammatory cytokines (IL- 1, IL-2, IL-6), INF-a, -y and TNF-a; T the activity of anti-inflammatory cytokines IL-10 and INF-a. There was a lack of pathogens' markers of the per- sistent viral infections in the blood serum on com- pleting the treatment.

*GP – group of children who were administered Proteflazid®; Gl – group of children who were administered Immunoflazid®; CG – comparison group; 1 – decrease; T – increase; N – normalization.

Conclusions

The analysis of scientific literature on the results of the clinical use of drugs derived from the active substance isolated from the plants *Deschampsia caespitosa* L. and *Calamagrostis epigeios* L. – Proteflazid® (drops) and Immunoflazid® (syrup) confirmed the availability of direct antiviral, immunomodulating and antioxidant properties. The use of drugs allow to eliminate the causative agent of the disease, normalises the imbalance of the child's immune system in a short time, reduces the timing of concomitant medications usage, thereby reducing the likelihood of adverse reactions of the child's organism. The high effectiveness of repeated courses of the drugs in children with recurrent diseases has been proved.

The results of systematic analysis of the scientific publications indicate a good clinical efficacy and a high profile of safety of Proteflazid® (drops) and Immunoflazid® (syrup) for the treatment of viral and viral-bacterial diseases in children, including of early age, which makes it possible to recommend these drugs for wide application in neonatology and paediatric practice.

References:

1. Katarzyna W. P., Włodzimierz G., Abramczuk E. et al. Non-influenza viruses in acute respiratory infections among young children. High prevalence of HMPV during the H1N1V.2009 pandemic in Poland. *Przegl Epidemiol*, 2014; 68: 627–632.
2. Kramarev S., Mikhailov V., Grynevych O. et al. Meta-Analysis of clinical trials results of efficacy and safety of the drug Proteflazid® in the treatment and prevention of human herpesvirus infection in children. *Asian Journal of Scientific and Educational Research*, 2016; №1(19): 766–783.
3. Porva Yu., Rybalko L., Palchykovs'ka L. Study of antiviral activity of a new plant origin preparation neoflazidum on a model of the hepatitis C virus. *Biopolymers and Cell*, 2015; 6: 465–472.
4. Starosyla D. B., Palchikovskaia L. I., Zavelevich M. P. Flavonoid compounds possessing broad spectrum of antiviral activities. 24 International Conference on Antiviral Research. – Sapporo, Japan. – April 16–19, 2012; 104.
5. World Health Organization. Cough and cold remedies for the treatment of acute respiratory infections in young children. WHO/FCH/CAH/01.02. WHO, 2001.

6. Антонов О. В., Антонова І. В., Турчанінов Д. В. Ідентифікація та аналіз причинно-наслідкових зв'язків у системі інфекційних захворювань – частота та характер вроджених вад розвитку. Епідеміологія та інфекційні хвороби, 2009; 5: 20–25.
7. Бекетова Г. В., Хайтович М. В., Гриневич О. Й. Імунофлазід у педіатрії: системний аналіз ефективності та безпеки застосування. Педіатрія. Східна Європа, 2014; 3(07): 141–152.
8. Бічний А. Г. Герпесвірусні інфекції у дітей. Діагностика, клініка та лікування. Роль у формуванні контингенту дітей, що часто хворіють. М: МАКС Прес, 2012; 144.
9. Веселій С. В., Климанський Р. П. Досвід застосування імунофлазиду в комплексному лікуванні різних форм герпесвірусної інфекції у дітей з вродженими вадами кишкової трубки. Український журнал хірургії, 2016; 2: 43–47.
10. Герасимов С. В., Чирун В. М., Вітковська Н. В. та ін. Ефективність Протефлазиду® при первинному герпетичному гінгівостоматиті у дітей раннього віку. Репродуктивне здоров'я жінки, 2006; 2: 142–144.
11. Годованець Ю. Д., Бабінцева А. Г. Обґрунтування призначення препарату ПРОТЕФЛАЗІД у комплексі лікування гіпоксичного ураження ЦНС у недоношених дітей в ранньому неонатальному періоді. Репродуктивне здоров'я жінки, 2005; 4(24): 96–199.
12. Годованець Д. В., Шунько Є. С. Досвід використання препарату ПРОТЕФЛАЗІД® у комплексному лікуванні нозологічних захворювань у дітей в ранньому неонатальному періоді. В монографії: Адаптація новонароджених дітей: функціональний стан гепатобіліарної системи в нормі та при перинатальній патології – Чернівці: БДМУ, 2007; 200–208.
13. Григорчук Н. В., Олійник В. С., Булат Л. М. Герпетична інфекція – деякі особливості її реабілітації у дітей грудного віку. Експериментальна і клінічна медицина, 2008; 4: 99–90.
14. Гриневич О. Й., Матяш В. І. Етіопатогенетична профілактика та лікування грипу та ГРВІ: нові можливості. Укр. мед. часопис, 2011; 4 (84): 20–26.
15. Зализюк А. А. Особливості сучасного перебігу негоспітальних пневмоній та їх лікування у дітей раннього віку. Автореферат дисертації на здобуття наукового ступеня кандидата медичних наук (14.01.10 – педіатрія). Харків, 2011.
16. Знаменська Т. К., Шевченко Л. І., Лошак О. О. та ін. Нові підходи до лікування внутрішньоутробної інфекції у новонароджених від матерів з бактеріально-вірусними мікст-інфекціями. Неонатологія, хірургія та перинатальна медицина, 2013; 2: 101–106.
17. Зубаренко О. В., Десятська Ю. В., Шевченко І. М. та ін. Імунофлазід у профілактиці інфекційних хвороб у дітей першого року життя. Сучасна педіатрія, 2015; 1: 123–127.
18. Зюзіна Л. С., Мизгіна Т. І. Застосування противірусних препаратів рослинного походження з метою лікування та профілактики рекурентних захворювань у дітей раннього віку. Проблемні питання діагностики та лікування дітей з соматичною патологією, 2015; 34–35.
19. Камінський В. В., Шалько М. М., Михайлов В. С. та ін. Оцінка ефективності препарату ПРОТЕФЛАЗІД® при лікуванні папіломавірусної інфекції: метааналіз результатів багаторічних клінічних досліджень. Медичні аспекти здоров'я жінки, 2015; 6(92): 5–14.
20. Кінаш Ю. М. Клініко-імунологічна характеристика дітей з рецидивуючим бронхітом та диференційований підхід до їх реабілітації і профілактики. Автореферат дисертації на здобуття наукового ступеня кандидата медичних наук (14.01.10 – педіатрія). Київ, 2010; 20.
21. Крамарьов С. О., Гриневич О. Й., Тонковід О. Б. та ін. Метааналіз результатів клінічних досліджень ефективності флавоноїдів при вірусних та вірусно-бактеріальних захворюваннях у дітей. Сучасна педіатрія, 2014; 5 (61): 1–7.
22. Крючко Т. А. Досвід застосування Протефлазиду у комплексній терапії нейроінфекції у дітей. Імунологія та алергологія, 2002; 3: 60.

23. Нагорна Н. В., Виноградов К. В. Ефективність застосування препарату ПРОТЕФЛАЗІД® при лікуванні герпесвірусних інфекцій у дітей із вродженими вадами серця. Перинатологія та педіатрія, 2007; 1(29): 76–79.
24. Овчаренко Л. С., Вертегел А. А., Андрієнко Т. Г. та ін. Нові шляхи санації внутрішньоклітинної інфекції у педіатрії. Сучасна педіатрія, 2004; 4 (5): 82–84.
25. Резніченко Н. А. Імуномодулятор у лікуванні перинатальних інфекцій. Питання експериментальної та клінічної медицини. Збірник статей, 2013; 17: 3–9.
26. Рибалко С. Л., Краснобаєв Є. А., Жеребцова Е. М. Сучасний стан проблеми грипу А (H1N1) 2009. Україна. Здоров'я нації, 2010; 3 (15): 169–178.
27. Сагатова М., Шамсієв Ф. М. Аналіз ефективності Протефлазиду при бронхолегеневій патології у дітей із герпесвірусною інфекцією. Медична імунологія: СПб РО РААКІ, 2009; 4–5: 396.
28. Токарчук Н. І., Старинець Л. С. Використання імунофлазиду для профілактики та лікування грипу і ГРВІ у дітей під час сезонного підвищення захворюваності. Сучасна педіатрія, 2012; 1(41): 123–127.
29. Турлібекова С. С. ПРОТЕФЛАЗІД у комплексній терапії вродженої цитомегаловірусної інфекції. Валеологія, 2011; 2: 164–166.
30. Усачова Е. В. Ефективність препарату ПРОТЕФЛАЗІД® при лікуванні дітей першого року життя з внутрішньоутробною цитомегаловірусною інфекцією. Репродуктивне здоров'я жінки, 2005; 2(22): 165–166.
31. Цимбаліста О. Л., Гаріджук Л. І. Динаміка маркерів запалення в дітей раннього віку, хворих на ускладнену пневмонію на тлі залізодефіцитної анемії. Перинатологія і педіатрія, 2013; 4(56): 33–36.
32. Шамсієв Н. Х., Мирсаліхова К. І., Алімова Д. П. та ін. Стан імунного статусу та ефективність Протефлазиду у комплексній терапії дітей з бронхолегеневою патологією, асоційованою з TORCH-інфекцією. Сучасна педіатрія, 2011; 2(36): 39–41.
33. Юліш Є. І., Баличевцева І. В., Гадецька С. Г. та ін. Нові підходи до профілактики та терапії гострих респіраторних інфекцій у дітей раннього віку, які проживають у будинках дитини. Сучасна педіатрія, 2009; 3(25): 15–18.
34. Юліш Є. І., Сорока Ю. А., Істраті М. А. та ін. Ефективність імунофлазиду у профілактиці гострих вірусних інфекцій у дітей дошкільного віку. Сучасна педіатрія, 2009; 4(26): 100–101.
35. Юліш Є. І., Чернишова О. Є., Кривущов Б. І. та ін. Протівірусні засоби в лікуванні та профілактиці гострих респіраторних захворювань у дітей, що часто і довго хворіють. Здоров'я дитини, 2011; 6: 95–99.
36. Юліш Є. І., Ярошенко С. Я. Діти, що часто хворіють, і тактика педіатра. Клінічна педіатрія, 2013; 6 (49): 102–108.

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2.3. META-ANALYSIS OF CLINICAL TRIALS RESULTS OF EFFICACY AND SAFETY OF THE DRUG PROTEFLAZID® IN THE TREATMENT AND PREVENTION OF HUMAN HERPESVIRUS INFECTION IN CHILDREN

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Abstract

18 publications involving 1 400 children between 2003 and 2014 years in a meta-analysis of clinical trials evaluating the efficacy and safety of the drug in the treatment of children PROTEFLAZID® with of human herpesvirus infection was included. The results show the high efficiency of application PROTEFLAZID® drug for the treatment of children with of human herpesvirus infection – CMV, EBV. Treatment with the drug contributes to the positive dynamics of the main clinical symptoms of disease and elimination of herpes viruses from the body.

Key words: meta-analysis, Epstein-Barr virus, cytomegalovirus, human herpesvirus infection, pediatrics, PROTEFLAZID®.

Introduction

Rationale. Human herpesvirus (HHV) infection is among the most common human viral diseases and holds a special place in children's infectious pathology.

During childhood, infection contamination occurs most often in contact, airborne or vertical ways, and the age of 6 years about 80 % of children are infected with the herpes simplex virus 1 and 2 (HSV) [1].

At present we know 8 antigenic serotypes of herpes viruses: herpes simplex virus 1 and 2 (HSV-1, HSV-2), cytomegalovirus (CMV; human herpes virus 5), Epstein-Barr virus (EBV, human herpes virus 4), varicella zoster virus (herpes zoster, human herpes virus 3), and human herpes viruses 6, 7 and 8. Herpes viruses are characterized by multisystemic tropism. The ability of pathogens to persist for a long time in the body by causing severe, persistent, chronic and latent forms of infection with periodic exacerbations is common for the HHV infection [21].

The problem of persistent intracellular infection is among the important problems in pediatrics due to high prevalence, long persistence in the body of the child, possible pathogenetic involvement in the formation of secondary immune deficiencies, proved influence of persistent

intracellular infection agents on the formation of a wide range of ante- and perinatal pathology [26].

A characteristic feature of modern infectious pathology is the growth of chronic infectious and inflammatory diseases, often emerging against the background of persistent infection process caused by following herpes viruses: HSV-1, HSV-2, CMV, EBV [27].

One of the most important forms of HHV infection is EBV caused infection. Antibodies to EBV have been found, according to published reports, in 40-60 % of children of the first three years of life, and in 80-90 % of adults [28].

In 90-95 % of cases, EBV causes the development of infectious mononucleosis (IM). IM in children, regardless of the pathogen and the age, is a symptom complex that includes long-term fever, systemic lymphadenopathy, acute tonsillitis, acute adenoiditis, hepatomegaly, splenomegaly, typical haematological changes in the blood in the form of leukocytosis, lymphocytosis, monocytosis, the presence of specific cells – atypical mononuclear cells or virocytes. The outcomes of IM according to different authors are recovery, asymptomatic carriage of viruses, persistent, latent, chronic form of infection, oncology lymphoproliferative process: lymphoma, nasopharyngeal carcinoma, leukoplakia of tongue, leukoplakia of mucous membranes of the oral cavity, stomach cancer, colon cancer, etc., autoimmune diseases: systemic lupus erythematosus (SLE), vasculitis, rheumatoid arthritis, Sjogren's syndrome, necrotizing enterocolitis (NEC), etc., chronic fatigue syndrome, splenic rupture, etc. [14].

In the human population CMV infection is widespread, it is a common factor for intrauterine fetal injury with the development of severe consequences and a cause of diseases in adults and children of different ages [7, 22, 29].

The variety of clinical manifestations of the disease is caused by the ability of CMV to replicate into almost all the body's cells: white blood cells, tissue macrophages, monocytes, endothelial, epithelial and neuronal cells, fibroblasts. CMV infection can occur acutely, chronically and latently, and manifest in congenital and acquired forms [29].

Long-term intracellular persistence of herpes viruses in the body of an infected child, multiple affinity to various organs and tissues and ability to reactivation lead to the need for treatment aimed to the suppression of infectious agent reproduction during acute manifestations, and the development of adequate immune response with long-term preservation [26].

One of the most effective means showing an expressed direct antiviral effect by inhibiting such virus specific enzymes as thymidine kinase (TK) and DNA polymerase, together with immunomodulatory action contributing to the induction of endogenous interferon, is the drug Proteflazid® (drops) and the drug Flavozid® (syrup) developed on the basis of drops and intended for use in the pediatrics (limited liability Scientific & Manufacturing Company “Ecopharm” Ltd, Ukraine). Active ingredients of the drug Proteflazid® are flavonoids that inhibit the synthesis of DNA viruses in infected cells through inhibition of activity of virus specific DNA polymerase and TK.

The drug Proteflazid® promotes the synthesis of endogenous interferons (IFN) such as α -IFN, γ -IFN to physiologically active level (without the occurrence of refractivity phenomenon), which increases nonspecific resistance to bacterial and viral infections, and normalizes immune status. The drug also prevents the accumulation of lipid peroxidation products (inhibits free radical processes) and is an apoptosis modulator, causing the death of infected cells [23, 28].

The drug Proteflazid® prevents the disease relapse and prolongs the period of remission.

It should be noted that the drug Proteflazid® shows high bioavailability, it is not toxic; it can be actively used in pediatrics and administered to children right after the birth and pregnant women.

Purpose of the trial.

Conduct the meta-analysis of clinical trials and evaluation of the clinical efficacy of the drug Proteflazid® in children with HHV infection (CMV, EBV).

Materials and Methods

Data of clinical trials that examine the effectiveness of the drug Proteflazid® in the treatment of HHV infection in children were received with the help of the Internet information retrieval systems. The list of selected 18 publications reflecting the effectiveness of the drug Proteflazid® in the treatment of HHV infection in 1,400 children during the period from 2003 to 2014 is presented in Table 1.

Software

In order to ensure the greater reliability and the accuracy, the calculations were conducted simultaneously in the two specialized statistical programs RevMan and SPSS.

Inclusion criteria:

The studies to be included in the meta-analysis shall meet the following criteria:

1. Controlled studies allowing for the control or the control group and focused on the study of the clinical efficacy of the drug Proteflazid® (Flavozid®) in the treatment of HHV infection (CMV, EBV) in children.

Table 1. The list of publications reflecting the efficacy of the drug Proteflazid® (Flavozid®) in the treatment of HHV infection in children

Author	General number of patients	Scope of application of the drug Proteflazid®	Clinical study results
<i>1. Publications reflecting the efficacy of the drug Proteflazid® (Flavozid®) in the treatment of EBV infection in children</i>			
Usachova E.V. [5]	38	Infectious mononucleosis caused by EBV	Significant reduction in clinical disease caused by EBV, positive dynamics of hemogram.
Chernyshova O.E. [24]	30	Immune and cytokine status in children at the background of infections caused by EBV	Relief of symptoms of acute infection, transition of chronic recurrent herpes infection into a latent form.
Kharchenko Yu.P. [8]	60	Infectious mononucleosis caused by EBV	Significant reduction in clinical disease caused by EBV
Kramarev S.A., Vigovskaya O.V. [2]	55	Chronic active EBV infection in children	Positive dynamics of clinical symptoms of the disease caused by EBV, arresting viral activity

Kramarev S.A., Vigovskaya O.V. [25]	35	Chronic EBV infection in children	Disappearance of markers of active viral replication from the blood: anti-EBV IgM VCA, EBV DNA after treatment
Borisova T.P. [17]	54	Hematuric form of chronic glomerulonephri- tis and related EBV infec- tion in children	Positive dynamics of symptoms, improvement in cytokine status
Kramarev S.A., Vigovskaya O.V. [14]	243	Infectious mononucleosis caused by EBV	Pronounced positive dynamics of indexes of T- and B-cell immunity, risk minimization of the tran- sition of acute stage into a chronic stage

2. Publications reflecting the efficacy of the drug Proteflazid® in the treatment of CMV infection in children

Usachova E.V. [7]	23	Intrauterine CMV infection in children	Positive dynamics of clinical symptoms of the disease, stabilization of specific immunological parameters
Reznichenko Yu.G. [15]	65	CMV infection in children with glomerulonephritis	Normalization of the immunity parameters
Znamenskaya T.K. [9]	200	Newborn babies of mothers with herpes and CMV infections	Improved clinical status of newborns, positive dynamics of cellular and humoral immunity
Turlibekova S.S. [22]	48	Congenital CMV infec- tion in children	Positive dynamics of clin- ical and laboratory data

3. Publications reflecting the efficacy of the drug Proteflazid® in the treatment of mixed HHV infection (HSV-1, HSV-2, CMV, EBV) in children

Kruchko T.A. [1]	62	HHV infection in children	Positive dynamics of clinical symptoms of the pathological process
Ershova I.B. [10]	86	Clinical and immunological features of viral meningoencephalitis in children	Prevention of relapse and acceleration of functional activity of children
Ovcharenko L.S. [13]	50	HHV and CMV infections in children with secondary immunodeficiency	load on the child with the pathology of immu- nity, reduction of virus-in- duced immunodeficiency
Kruchko T.O. [4]	58	Prevention of viral fatigue syndrome in children with HHV infection	Restoring function of the affected organs, easier relapses

Antonova N.M. [19]	93	Acute oral herpes and prevention of relapses of HHV infection in children	Improved immunological status of children, positive dynamics of cellular and humoral immunity
Yulish E.I. [3]	130	Prolonged low-grade fever in children with HHV infection	Significant reduction in clinical manifestations of concurrent infection, resistant anti-relapse effect

2. It is necessary to confirm the clinical diagnosis of diseases identified in children.

3. Published data on the effectiveness of the drug Proteflazid® (Flavozid®) are complete.

Studies that did not meet the stated criteria were excluded from the metaanalysis.

Taking into account the aforementioned criteria, the meta-analysis includes 18 controlled clinical studies of the effectiveness of the drug Proteflazid® (Flavozid®) involving 1400 children with HHV infection.

All children before the beginning of the treatment and after the treatment were thoroughly examined, including conventional clinical and laboratory tests, ultrasound study of cellular and humoral immunity. Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) were used as main methods of diagnosis.

The meta-analysis of clinical trials reflecting the results of the effectiveness of the drug Proteflazid® (Flavozid®) in the treatment and prevention of HHV infection in children was conducted by the following statistically significant indexes:

1. Dynamics of EBV clinical symptoms in children after the treatment of HHV infection with the drug Proteflazid® (Flavozid®) at the completion of treatment:

- The detection rate of lymphadenopathy in children with EBV infection.
- The detection rate of hepatomegaly in children with EBV infection.
- The detection rate of splenomegaly in children with EBV infection.

2. Dynamics of CMV infection markers in the blood of children after a course of treatment with the drug Proteflazid®:

- The detection rate of CMV DNA by the use of the PCR in children with CMV infection.

Clinical trials results as for the aforementioned parameters are presented in tables 2-5.

Table 2. The detection rate of lymphadenopathy in children with EBV infection

Author	Experimental group		Control group	
	The number of children treated with Proteflazid® (Flavozid®) who showed lymphadenopathy	General number of patients	The number of children not treated with Proteflazid® (Flavozid®) who showed lymphadenopathy	General number of patients
Usachova O.V., 2005	3	17	11	21
Kharchenko Yu.P., 2007	2	30	10	30

Kramarev S.A., 2012	3	5	5	5
Kramarev S.A., 2014	30	60	44	50
Total	38	112	70	116

228 children with EBV infection were studied by the parameter “Detection rate of lymphadenopathy in children with EBV infection” after the treatment with the drug Proteflazid® (Flavozid®), 112 of them are from the experimental group, and 116 – from the control group.

Table 3. The detection rate of hepatomegaly in children with EBV infection

Author	Experimental group		Control group	
	The number of children treated with Proteflazid® (Flavozid®) who showed hepatomegaly	General number of patients	The number of children not treated with Proteflazid® (Flavozid®) who showed hepatomegaly	General number of patients
Usachova O.V., 2005	6	17	8	21
Kharchenko Yu.P., 2007	6	30	19	30
Kramarev S.A., Vigovskaya O.V., 2012	5	5	5	5
Kramarev S.A., Vigovskaya O.V., 2014	19	60	40	60
Total	36	112	72	116

228 children with EBV infection were studied by the parameter “Detection rate of hepatomegaly in children with EBV infection” after the treatment with the drug Proteflazid® (Flavozid®), 112 of them are from the experimental group, and 116 – from the control group.

Table 4. The detection rate of splenomegaly in children with EBV infection

Author	Experimental group		Control group	
	The number of children treated with Proteflazid® (Flavozid®) who showed splenomegaly	General number of patients	The number of children not treated with Proteflazid® (Flavozid®) who showed splenomegaly	General number of patients
Usachova O.V., 2005	7	17	13	21
Kharchenko Yu.P., 2007	2	30	18	30
Kramarev S.A., Vigovskaya O.V., 2012	4	5	5	5
Total	13	52	36	56

108 children with EBV infection were studied by the parameter “Detection rate of splenomegaly in children with EBV infection” after the treatment with the drug Proteflazid®, 52 of them are from the experimental group, and 56 – from the control group.

Table 5. The detection rate of CMV DNA detection by the use of the PCR in children with CMV infection

Author	Experimental group		Control group	
	The number of children treated with Proteflazid® who showed CMV DNA	General number of patients	The number of children not treated with Proteflazid® who showed CMV DNA	General number of patients
Reznichenko Yu.G., 2007	3	14	7	10
Turlibekova S.S., 2011	0	23	5	25
Total	3	37	12	35

72 children with EBV infection were studied by the parameter “Detection rate of CMV DNA detection by the use of the PCR in children with CMV infection” after the treatment with the drug Proteflazid®, 37 of them are from the experimental group, and 35 – from the control group.

Results and Discussion

Assessment of the effectiveness of the drug Proteflazid® (Flavozid®) in the treatment of EBV infection in children.

Fig. 1 shows a Forest plot for the meta-analysis results on the basis of OR of the detection rate of lymphadenopathy in children with EBV infection after the treatment with the drug Proteflazid® (Flavozid®) in experimental and control groups.

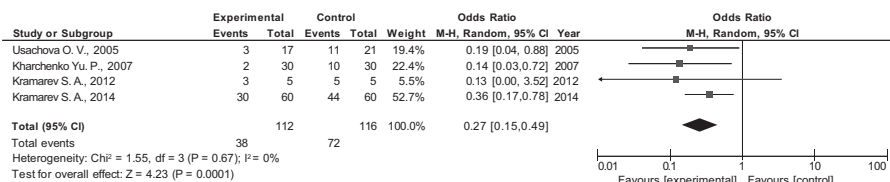


Fig. 1. The results of meta-analysis by identifying the OR of detection rate of lymphadenopathy in children with EBV infection

χ -square criterion ($P=0.67$) and I^2 -test ($I^2=0\%$) indicate the homogeneity of these studies, so a model with fixed effect has been chosen. P-value of the Fisher test ($P=0.0001$) shows the importance of the selected effect.

Odds ratio ($OR=0.27$) indicates that the probability of the detection rate of lymphadenopathy in children with EBV infection after the treatment with the drug Proteflazid® (Flavozid®) in the experimental group is 3.7 times lower than in the control group.

The study conducted by Kramarev S.A., Vigovskaya O.V. (2014) has the greatest weight. They note that the drug Proteflazid® (Flavozid®) has a high clinical efficacy, an expressed immunocorrecting and antiviral activity in treatment of infectious mononucleosis of EBV etiology in children [14]. The study conducted by Kharchenko Yu.P. et al. (2007) indicates

that the complex therapy with the drug Proteflazid® reduces the duration of the febrile period, the severity of infectious intoxication, the duration of symptoms of the lymphatic system damage [8].

Fig. 2 presents a Forest plot of the results of the meta-analysis by identifying the OR of the detection rate of hepatomegaly in children with EBV infection after treatment with the drug Proteflazid® (Flavozid®) in experimental and control groups.

χ -square test ($P = 0.11$) and I^2 -test ($I^2= 54 \%$) indicate an insignificant heterogeneity of these studies, so a model with random effect has been chosen. P-value of the Fisher test ($P = 0.007$) shows the importance of the selected effect.

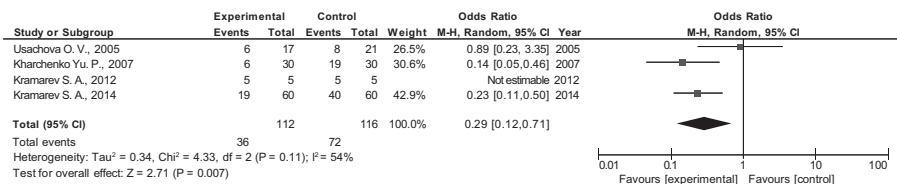


Fig. 2. The results of the meta-analysis by identifying the OR of the detection rate of hepatomegaly in children with EBV infection

Odds ratio ($OR=0.29$) indicates that the probability of the detection rate of hepatomegaly in children with EBV infection after treatment with the drug Proteflazid® in the experimental group is 3.45 times lower than in the control group.

The study conducted by Kramarev S.A., Vigovskaya O.V. (2014) has the greatest weight. They note that the drug Proteflazid® (Flavozid®) against the background of basic therapy of infectious mononucleosis in children increases the efficacy of treatment in the acute stage and minimizes the risk of progression of the disease in the acute phase into a protracted chronic disease [14]. The study conducted by Kharchenko Yu.P. et al. (2007) indicates that the complex therapy with the drug Proteflazid® reduces the frequency of hepatosplenomegaly [8]. Usacheva O.V. et al. (2005) noted a quicker arresting of hepatocyte cytolysis syndrome which is a manifestation of the complex course of infectious mononucleosis in children [5].

Fig. 3 presents a Forest plot of the results of the meta-analysis by identifying the OR of the detection rate of splenomegaly in children with EBV infection after treatment with the drug Proteflazid® (Flavozid®) in experimental and control groups.

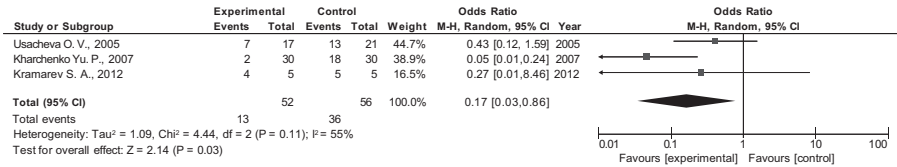


Fig. 3. The results of the meta-analysis by identifying the OR of the detection rate of splenomegaly in children with EBV infection

χ -square test ($P=0.11$) and I^2 -test ($I^2=55 \%$) indicate an insignificant heterogeneity of these studies, so a model with random effect has been chosen. P-value of the Fisher test ($P=0.03$) shows the importance of the selected effect.

Odds ratio (OR=0.17) indicates that the probability of the detection rate of splenomegaly in children with EBV infection after the treatment with the drug Proteflazid® (Flavozid®) in the experimental group is 5.88 times lower than in the control group.

As for this sampling, the study conducted by Usacheva O.V. et al. (2005) demonstrated that the inclusion of the drug Proteflazid® in the complex therapy of infectious mononucleosis of EBV etiology in children leads to the positive dynamics of clinical symptoms of the disease and the blood count [5].

Assessment of the effectiveness of the drug Proteflazid® in the treatment of CMV infection in children.

Fig. 4 presents a Forest plot of the results of the meta-analysis by identifying the OR of the detection rate of CMV DNA in children with CMV infection by the use of the PCR after the treatment with the drug Proteflazid® at the completion of treatment in experimental and control groups.

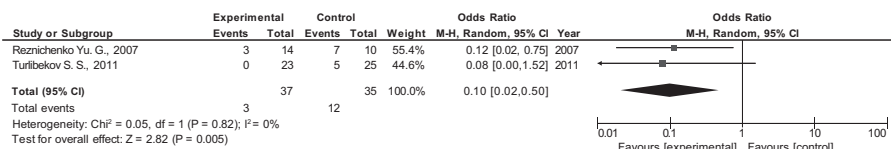


Fig. 4. The results of the meta-analysis by identifying the OR of the detection rate of CMV DNA in children with CMV infection by the use of the PCR

χ -square test ($P=0.82$) and I^2 -test ($I^2=0\%$) indicate the heterogeneity of these studies, so a model with fixed effect has been chosen. P-value of the Fisher test ($P=0.005$) shows the importance of the selected effect.

Odds ratio (OR=0.10) indicates that the probability of the detection rate of CMV DNA in children with CMV infection by the use of the PCR after the treatment with the drug Proteflazid® at the completion of treatment in the experimental group is 10 times lower than in the control group.

The study conducted by Reznichenko Yu.G. et al. (2007) has the greatest weight, it states that the use of the drug Proteflazid® in most cases ensures the CMV DNA elimination, improves the body's metabolism and the immune function [15].

Turlibekov S.S. (2011) concludes that the drug Proteflazid® enhances the innate immunity by increasing the level of endogenous interferon, which improves the resistance of the body not only against viruses but against bacteria as well [22].

Sensitivity Analysis

The sensitivity analysis was carried out in such a way as to assess the influence of each individual study on combined data by omitting individual research. The results of the sensitivity analysis showed that no individual study influenced significantly the combined data, which indicates statistically reliable results.

Assessment of publication bias

Funnel plot was used for assessing the bias of publications included into the study (Fig. 5-8). Almost all of the ES values in Fig. 5-8 are within the funnel, which indicates the absence of bias.

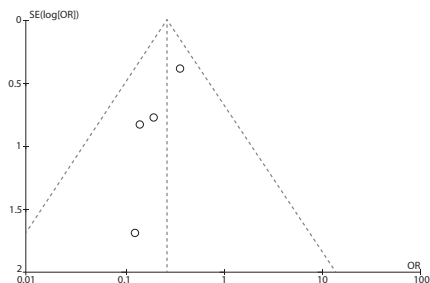


Fig. 5. Funnel plot for the index “The detection rate of lymphadenopathy in children with EBV infection”

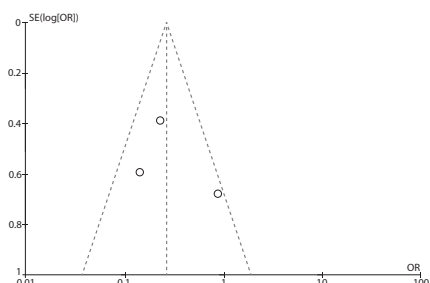


Fig. 6. Funnel plot for the index “The detection rate of hepatomegaly in children with EBV infection”

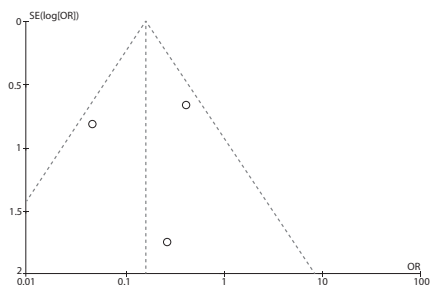


Fig. 7. Funnel plot for the index “The detection rate of splenomegaly in children with EBV infection”

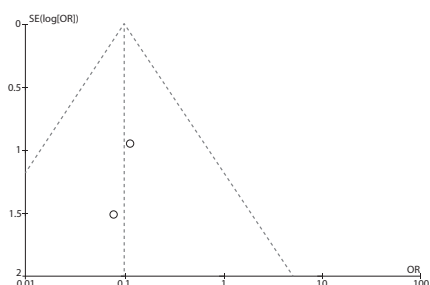


Fig. 8. Funnel plot for the index “The detection rate of CMV DNA detection by the use of the PCR in children with CMV infection”

Conclusion

This meta-analysis allowed increasing the evidence base for the effectiveness and the safety of the drug Proteflazid® for treatment of viral and concurrent infections in children.

The meta-analysis of clinical studies included 18 controlled clinical studies covering 1400 children for assessing the clinical effectiveness of the drug Proteflazid® in the treatment of children with HHV infection (CMV, EBV).

The meta-analysis has been conducted according to 4 indexes:

1. Detection of clinical symptoms of HHV infection in children after the treatment with Proteflazid® (Flavozid®) at the completion of treatment:
 - The detection rate of lymphadenopathy in children with EBV infection.
 - The detection rate of hepatomegaly in children with EBV infection.
 - The detection rate of splenomegaly in children with EBV infection.
2. Dynamics of CMV infection markers in the blood of children after a course of treatment with the drug Proteflazid®:
 - The detection rate of CMV DNA detection by the use of the PCR in children with CMV infection.

This meta-analysis confirms the effectiveness and the safety of the drug Proteflazid® in clinical use based on the following obtained results: in administering the drug Proteflazid® in children with EBV and CMV infection, (experimental group) positive dynamics of clinical symptoms of disease was observed at the stage of completion of the study:

- Reduction in the probability of detection rate of clinical symptoms of EBV infection: lymphadenopathy in the experimental group was 3.7 times lower than in the control group; hepatomegaly in the experimental group was 3.45 times lower than in the control group; splenomegaly in the experimental group was 5.88 times lower than in the control group.

- during treatment, the probability of the detection rate of CMV DNA in children with CMV infection by the use of PCR was 10 times lower than in children who did not take the drug Proteflazid® (control group).

Meta-analysis of clinical trials shows a high efficiency of the drug Proteflazid® in the treatment of children with HHV infection (CMV, EBV). The use of the drug in the treatment of HHV infection (including its mixed forms) contributes to the positive dynamics of the main clinical symptoms of disease – reduction in the detection rate of lymphadenopathy, splenomegaly and hepatomegaly and elimination of herpes viruses from the body of the child.

Given its high tolerability, high safety profile, combined effect on many elements of viral infections, the drug Proteflazid® can be recommended as an effective and safe antiviral agent for the treatment and prevention of diseases caused by HHV infection (CMV, EBV) in children.

References:

1. Крючко Т. О. Актуальні питання лікування герпетичної інфекції у дітей / Крючко Т. О., Ю. М. Кінаш // Перинатологія та педіатрія. – 2006. – №3 (27). – С. 60–63.
2. Використання препарату Флавозід у комплексному лікуванні хронічної активної Епштейна-Барр вірусної інфекції у дітей / С. О. Крамарьов [та ін.] // Сучасна педіатрія. – 2008. – №3 (20). – С. 111–114.
3. Юліш Є. І. Тривалий субфебрилітет у дітей. Можливі причини та підходи до терапії / Юліш Є. І., О. Є. Чернишова, Ю. Л. Сорока // Сучасна педіатрія. – 2011. – №1 (35). – С. 67–72.
4. Крючко Т. О. До питання профілактики поствірусного синдрому втомлюваності у дітей з герпетичною інфекцією / Крючко Т. О., Ю. М. Кінаш // Перинатологія та педіатрія. – 2008. – №1 (33). – С. 111–115.
5. Досвід використання препарату ПРОТЕФЛАЗІД® при інфекційному мононуклеозі у дітей / О. В. Усачова [та ін.] // Репродуктивне здоров'я жінки. – 2005. – №4. – С. 192–194.
6. Герасимов С. В. Етіотропне лікування Флавозідом гострого герпетичного гінгівостоматиту у немовлят і дітей раннього віку / С. В. Герасимов // Перинатологія та педіатрія. – 2007. – №2 (30). – С. 75–78.
7. Усачова О. В. Ефективність препарату ПРОТЕФЛАЗІД® при лікуванні дітей першого року життя з внутрішньоутробною цитомегаловірусною інфекцією / О. В. Усачова // Репродуктивне здоров'я жінок. – 2005. – №2 (22). – С. 165–166.
8. Харченко Ю. П. Застосування препарату Флавозід при інфекційному мононуклеозі у дітей / Ю. П. Харченко, Г. А. Шаповалова // Сучасна педіатрія. – 2007. – №4 (17). – С. 115–118.
9. Звіт про проведення клінічного дослідження «Вивчення ефективності та переносимості препарату Флавозід у новонароджених дітей від матерів з герпесвірусною інфекцією» (Звіт) / ДУ «Інститут педіатрії, акушерства та гінекології АМН України» – К., 2008. – 35 с.

10. Єршова І. Б. Клініко-імунологічне обґрунтування застосування лаферону та Протефлазиду під час лікування серозних менінгітів в дітей віком / І. Б. Єршова, Т. А. Гончарова, Н. П. Скородумова // Проблеми екологічної та медичної генетики і клінічної імунології: зб. наук. праць Ювіл. вип., присвяч. 60-річчю з дня народж. Г.М. Драннік. – К. та ін., 2003. – Вип. 1(47). – С. 83–91.
11. Юліш Є. І. Комплекс клініко-лабораторних симптомів при рекурентних обструктивних бронхітах на тлі персистуючої інфекції у дітей раннього віку. Ефективність диференційованої терапії / Є. І. Юліш, С. І. Вакуленко // Сучасна педіатрія. – 2010. – №3 (31). – С. 1–4.
12. Андрієць О. О. Комплексне лікування запальних захворювань зовнішніх статевих органів та піхви у дівчаток і підлітків з використанням Протефлазиду / О. О. Андрієць // Репродуктивне здоров'я жінок. – 2005. – №2 (22). – С. 168–173.
13. Лікування герпетичної та цитомегаловірусної інфекції у дітей з другорядним імунodefіцитом / Л. С. Овчаренко [та ін.] // Перинатологія та педіатрія. – №3 (27). – 2006. – С. 61–65.
14. Лікування інфекційного мононуклеозу у дітей / Крамарьов С. О., О. В. Виговська, Н. М. Тарадій, О. Й. Гриневич // Сучасна педіатрія. – 2014. – №4(60). – С. 55–62.
15. Лікування цитомегаловірусної інфекції у дітей з гломерулонефритом / Резніченко Ю. Г. [та ін.] // Пленум правління Української асоціації нефрологів: Тези доп. – Одеса. – 2007. – С. 74–75.
16. Нові підходи до лікування внутрішньоутробної інфекції у новонароджених від матерів з бактеріально-вірусними мікст-інфекціями / Т. К. Знаменська [та ін.] // Неонатологія, хірургія та перинатальна медицина. – 2013. – №2(8). – С. 101–106.
17. Борисова Т. П. Нові підходи до терапії дітей з гематуричною формою хронічного гломерулонефриту та супутньою хронічною Епштейна-Барр вірусною інфекцією / Т. П. Борисова, Є. М. Толчєннікова // Проблемні питання педіатрії та вищої медичної освіти: зб. наук. праць (VII випуск). – Донецьк: Норд-Прес, 2013. – С. 425.
18. Особливості терапії рецидивуючого обструктивного бронхіту у дітей раннього віку, інфікованих внутрішньоклітинними патогенами / Юліш Є. І., І. В. Баличевцева, С. Г. Гадецька, С. І. Вакуленко // Сучасна педіатрія. – 2007. – №3 (16). – С. 175–178.
19. Антонова Н. М. Особливості медичного використання препарату Флавозід для лікування гострого герпесу порожнини рота та профілактики рецидивів герпетичної інфекції у дітей / Н. М. Антонова, Н. О. Савичук // Збірник наукових праць співробітників НМАПО імені П. Л. Шупика. – К.: НМАПО ім. П. Л. Шупика, 2010. – №19. – С. 326–335.
20. Андрієць О. О. Особливості перебігу вульвовагінітів вірусної етіології у дівчат препубертатного та пубертатного віку / О. А. Андрієць, І. Б. Вовк, О. М. Юзько // Буковинський медичний вісник. – 2014. – №1. – С. 15–18.
21. Крамарьов С. О. Звіт про проведення клінічного дослідження ефективності та переносимості препарату Флавозід у дітей з герпесвірусними інфекціями / Крамарьов С. О., О. В. Виговська // Клінічна імунологія. Алергологія. Інфектологія – 2012. – №4 (53). – С. 1–8.
22. Турлібекова С. С. ПРОТЕФЛАЗИД® у комплексній терапії вродженої цитомегаловірусної інфекції / С. С. Турлібекова // Валеологія. – 2011. – №2. – С. 164–166.
23. Стан імунного статусу та ефективність Протефлазиду в комплексній терапії дітей з бронхолегеневою патологією, асоційованою з TORCH інфекцією / Ф. М. Шамсієв, Н. Х. Мирсалихова, К. І. Алімова, Д. П. Таджиханова // Сучасна педіатрія. – 2011. – №2 (36). – С. 39–41.
24. Чернишова О. Є. Характер змін здоров'я дітей раннього віку, стан їх імунного та цитокінового статусу на тлі різної течії інфекції, спричиненої вірусом Епштейна-Барр / О. Є. Чернишова, Є. І. Юліш, Л. А. Іванова // Лікарська практика. – 2007. – №1 (55). – С. 24–28.

25. Крамарьов С. О. Хронічні форми Епштейна-Барр вірусної інфекції у дітей: сучасні підходи до діагностики та лікування / Крамарьов С. О., О. В. Виговська // Сучасна педіатрія. – 2008. – №2 (19). – С. 103–108.
26. Нагорна Н. В. Ефективність препарату ПРОТЕФЛАЗІД® при лікуванні герпесвірусних інфекцій у дітей із вродженими вадами серця / Н. В. Нагорна, К. В. Виноградов // Перинатологія та педіатрія. – 2007. – №1 (29). – С. 76–79.
27. Чернишова О. Є. Лікування та реабілітація дітей з різним перебігом герпесвірусної інфекції / О. Є. Чернишова // Науково-практична конференція, присвячена 75-річчю Донецького державного медичного університету ім. М. Горького «Внутрішньоклітинні інфекції та стан здоров'я дітей у ХХІ столітті» (25–26 жовтня 2005): Тези дод. – Донецьк, 2005. – С. 9.
28. Крамарьов С. О. Досвід використання препарату Флавозід при Епштейна-Барр вірусній інфекції у дітей / Крамарьов С. О., О. В. Виговська // Сучасна педіатрія. – 2011. – №5 (39). – С. 1–5.
29. Цитомегаловірусна інфекція у дітей / Юліш Є. І. [та ін.] // Новини медицини та фармації. Антимікробна та противірусна терапія. – 2008. – №236 (Тематичний номер). – С. 12–19.

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2.4. PROTEFLAZID: SPECIFIC ACTIVITY IN EPSTEIN-BARR VIRUS INFECTION IN A PRECLINICAL STUDY, EFFICACY AND SAFETY IN THE CLINIC (SYSTEMATIC REVIEW)

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Abstract

We analyze the specific anti-EBV (Epstein-Barr virus)-activity of the active ingredient Proteflazid extract and preparations based on it: PROTEFLAZID® (drops), Flavozid® (syrup). Preclinical studies have shown that the active ingredient blocks DNA polymerase and thymidine kinase in EBV infected cells, induces the synthesis of endogenous α - and γ -interferons, has antioxidant activity and also apoptosis-modulating action. Efficacy of drugs for diseases caused by the Epstein-Barr virus has been clinically proven. The safety and efficacy of PROTEFLAZID® (drops) and Flavozid® (syrup) in children (755 patients) and adult (592 patients) in the clinic has been confirmed. The unidirectional positive therapeutic effect in 16 clinical observations in the period from 2002 to 2014 was noted.

Key words: PROTEFLAZID®, Flavozid®, antiviral preparations, Epstein-Barr virus infection, infectious mononucleosis, treatment.

Introduction

Relevance. One of the most common forms of herpesvirus is infection caused by the Epstein-Barr virus (EBV, herpes virus type 4), which belongs to the subfamily Gamma herpes virinae [1, 2]. The rate of infection with EBV in the adult population is almost 90-100%, and in children, according to various authors, from 50% to 80% [3]. The time of first contact with the virus depends on social conditions: in developing countries or socially disadvantaged families, most children are infected by the age of 3, and the entire population by adulthood; in developed countries, EBV infection may occur later. Primary EBV infection usually remains unnoticed, occurring asymptotically or in the form of a moderate increase in the activity of liver enzymes [4, 5]. Only 1/6 of patients develop clinical manifestations of infectious mononucleosis, an acute form of Epstein-Barr virus (EBV) infection [6, 7]. A feature of EBV infection is its tendency to relapse, which is observed in 1/3 of patients, and the development of a chronic form of the disease [8].

The most attention of specialists is attracted by the acute form, which usually has a favorable course, but does not end with the complete elimination of the pathogen. The further disease becomes latent, which may not manifest itself in any way throughout a person's life, but in the presence of immunodeficiency or other adverse factors, reactivation of the infection with severe damage to organs and systems is possible, which is clinically manifested by meningitis, encephalitis, polyradiculoneuritis, hepatitis, etc. [8, 9]. In 10-25% of cases, acute EBVI can have adverse consequences with the subsequent formation of lymphoproliferative, oncohematological diseases, chronic fatigue syndrome, EBV-associated hemophagocytic syndrome, autoimmune pathology, etc. [10, 11, 12].

EBV infection is an immunological paradox: The virus infects B lymphocytes and persists in these cells, which are representatives of the immune system [13, 14]. Clinical manifestations and pathogenesis of infection are the result of an immune duel between infected B lymphocytes and cytotoxic lymphocytes. EBV exerts a powerful immunosuppressive effect, causing a violation of the immune response [1, 2, 3, 15]. The formation of immunity in EBVI is a complex and multicomponent process and includes both a compensatory (response to a persistent antigen) effect and inadequate regulation of the virus-specific immune response [15, 16]. In conditions of weakened immunological control, not only is complete elimination of the intracellularly located virus impossible, but also favorable conditions are created for the spread of the virus from cell to cell via intercellular bridges or extracellularly [16, 17]. The virus can infect and persist for a long time in immunocompetent cells, which eliminates the possibility of eliminating the pathogen and complete recovery of the body, creating conditions for the development of immunopathological reactions with damage to various organs and systems and clinical manifestations of immunodeficiency [9, 10, 11, 16, 17].

Numerous studies confirm that EBV is responsible for malignant transformation of infected cells. It has been proven that such types of cancer as nasopharynx, larynx, circulatory system, kidney, genitals, nervous system are often associated with this virus [9, 10]. The mechanism of action of EBV is that the LMP-1 protein, which is part of the virus, not only blocks apoptosis but also helps pathogenic cells to grow, multiply, and even migrate [18, 19]. At the molecular level, the affected cells do not undergo apoptosis, which is why the tumor process develops [20]. One of the targets activated by the viral protein is the epidermal growth factor receptor (EGFR). This receptor affects the cell in the same way as the viral protein LMP-1. That is, the virus provokes and supports oncogenesis in the body of an infected person both by using its protein LMP-1 and the cellular protein EGFR [10, 11, 12, 13, 20]. If EGFR, due to its functions, triggers oncogenesis, then LMP-1 (in addition to the above functions) blocks immune system cells and supports angiogenesis (formation and growth of new vessels), creating favorable conditions for tumor growth and development [18, 19, 20].

Approaches to the treatment of EBVI are based mainly on clinical data. In terms of rational use, priority is given to nucleoside drugs (acyclovir, ganciclovir, valganciclovir, valganciclovir), despite the fact that they have a significant number of contraindications. At the same time, during the treatment process, practicing physicians note the insufficient effectiveness of monotherapy with these drugs and try to potentiate it with specific immunoglobulins and interferons, which in turn lead to even more serious violations of the body's homeostatic capabilities and the development of persistent autoimmune processes.

Due to different approaches to EBVI therapy, the lack of highly effective and safe treatment algorithms, and the development of resistance to certain drugs, the issue of creating new effective and fairly safe EBVI therapy regimens is becoming relevant [21, 22, 23].

Antiviral drugs, the active ingredient of which is flavonoids of the PROTEFLAZID® extract: PROTEFLAZID® (drops) and Flavozid® (syrup; ed. note: since 2019, the release of this dosage form has been discontinued), manufactured by “SMC “Ecopharm” Ltd. (UKRAINE) [24, 25].

PROTEFLAZID® extract is obtained by the technology of alcohol extraction (96% ethanol) of plant raw materials (wild herbs – *Calamagrostis epigeios* L. and *Deschampsia caespitosa* L.). The drug contains flavonoids (flavone and flavonol glycosides) isolated from cereals, presented in the form of stable molecular complexes: tricin, apigenin, luteolin, and quercetin [25].

Objective of the study

To confirm the efficacy and safety of medicinal products based on PROTEFLAZID® extract [(PROTEFLAZID® (drops), Flavozid® (syrup)] in the treatment of diseases caused by the Epstein-Barr virus through a systematic analysis of preclinical and clinical studies in children and adults.

Materials and research methods

Scientific publications with preclinical (4 papers) and clinical (16 papers) studies; systematic analysis.

Results and discussion

Preclinical studies

S. L. Rybalko (2003), S. D. Zagorodnya et al. (2009), in studies of cytotoxicity and specific anti-EBV activity, showed the effectiveness of the active ingredient of the PROTEFLAZID® extract in vitro in a culture of Raji lymphoblastoid cells (human B-lymphocytes) against Epstein-Barr virus. Antiviral activity was assessed by PCR by reduction of the percentage of genomic equivalents of EBV DNA per cell. The data obtained indicate a dose-dependence between the concentration of the PROTEFLAZID® extract and EBV reproduction.

The use of the drug at a concentration of 0.1 µg/ml leads to a 50% reduction in the number of genomic equivalents of EBV DNA per cell [24, 27].

It was confirmed that the PROTEFLAZID® extract had a relatively high selectivity index (SI). For different solvents and schemes of use of PROTEFLAZID® extract, SI is from 250 to 1500 [24, 26, 27]. According to methodological recommendations from preclinical studies, substances with SI values equal to or higher than 16 can be considered highly active and promising for further study [29].

The established fact of the effectiveness of the active substance of PROTEFLAZID® extract against the Epstein-Barr virus in Raji cell culture allowed the drug to be classified as active and promising for the treatment of diseases caused by this virus [24, 27]. As shown in the following studies by S. L. Rybalko (2010), D. B. Starosila (2011), flavonoids, which are part of PROTEFLAZID® extract, are able to inhibit the synthesis of virus-specific enzymes DNA polymerase and thymidine kinase in virus-infected cells [25, 26].

It should be noted that a number of preclinical studies have confirmed the ability of the active ingredient of PROTEFLAZID® extract to render antiviral effects on other herpesviruses, in particular on HSV-1 and HSV-2 [24, 25, 26, 27].

In addition to antiviral activity, the active substance PROTEFLAZID® also exhibits antioxidant activity: it increases the resistance of cells to free radical stress during infections, reduces the negative effects of drug chemotherapy, and promotes the body's adaptation to adverse environmental conditions. It has been proven that the drug inhibits the intensity of free radical processes induced by hydrogen peroxide by more than 2 times. At the cellular level, it has been shown that PROTEFLAZID® inhibits the generation of superoxide radical anion to almost zero within 24 hours of drug administration, i.e., maintains the antioxidant status of cells [24].

Kramarev S. O. et al. (2013) proved that in the pathogenesis of EBVI chronicity, a violation of apoptosis mechanisms is of great importance [28]. PROTEFLAZID® also has apoptosis-modulating activity – it contributes to the primary prevention of the occurrence of oncological diseases on the background of chronic (latent) viral infections. The active ingredient of PROTEFLAZID® extract restores the ability of virus-infected cells to undergo apoptosis by activating caspase 9, reducing the activity of proliferative processes in mutated cells [25].

In terms of acute toxicity, herbal antiviral drugs containing flavonoids (PROTEFLAZID®, Flavozid®) belong to the class of relatively safe substances. In preclinical studies, no teratogenic, mutagenic, or embryotoxic effects were detected [24, 25, 26].

Clinical studies

In clinical practice, the medicinal product PROTEFLAZID® (drops) has been used for the treatment of EBVI since 2002. Matyash V. I. et al. (2002) developed appropriate schemes for etiopathogenetic therapy of severe forms of herpetic infection. Based on the results of the studies, conclusions were drawn about the feasibility of using complex etiotropic therapy, including PROTEFLAZID®, in the treatment of severe forms of herpetic infection, including EBVI, which allows achieving a stable effect on restoring the function of the affected organs and systems [30].

T. O. Nikiforova et al. (2004) conducted a study of the clinical and immunological effectiveness of the medicinal product PROTEFLAZID® in patients with confirmed EBVI. As the results of the observation showed, the drug contributed to the rapid disappearance of intoxication and lymphoproliferative symptoms, restoration of liver function, and significantly reduced the level of IgG in the blood [31].

A. I. Gley (2005, 2008) demonstrated the effectiveness of the drug PROTEFLAZID® as an antiviral and immunomodulatory agent for the treatment of EBV-induced infectious mononucleosis (IM). It was shown that the use of the drug for 2 months, according to the scheme described in the instructions for use, reduces the duration of viremia, the duration of the disease, and prevents the formation of a chronic form of the disease [32, 33].

E. V. Usachiova et al. (2005) in a study of the clinical efficacy and tolerability of the medicinal product PROTEFLAZID® in infectious mononucleosis (IM) in children confirmed the effectiveness of the drug in complex therapy, which was manifested by positive dynamics in the clinical symptoms of the disease and hemogram indicators. Against the background of taking PROTEFLAZID®, a faster cessation of the hepatocyte cytolysis syndrome was noted, which characterizes the manifestation of the severity of the disease [34].

O. L. Panasiuk (2006) based on the results of a comprehensive study established the prevalence of associations of herpesvirus infections, which include viruses with different sensitivity to antiviral drugs. Under these conditions, the effects of PROTEFLAZID® on the

clinical course of the disease, immunological reactivity, and dynamics of interferon induction in patients with various associations of herpesviruses, including EBV, were studied. It was proven that PROTEFLAZID® reduced toxicity and mitigated the effectiveness of antiviral therapy; the relationship between the dynamics of interferon conversion and the terms of positive clinical dynamics was established. According to the results of the studies, indications and regimens for taking the medicinal product PROTEFLAZID® with anti-relapse purposes were developed. It was proven that long-term use of the drug PROTEFLAZID® during the convalescence period was safe and reduced the frequency of virus-positive and virus-negative relapses by 1.9 times and by 1.3 times. A comparative assessment of the effectiveness of complex therapy regimens based on various combinations of the drug PROTEFLAZID® (drops) and other agents was also given [35].

Yu. P. Kharchenko, G. A. Shapoval (2007) performed a comparative clinical study, in which they evaluated the effectiveness of the use of syrup in the complex therapy of IM in children, taking into account the clinical and immune status. The results of the study showed that the use of the drug allowed to reduce the average duration of hospital stay by 1.5 times, reduced the duration of fever, reduced the severity of intoxication syndrome, and shortened the duration of lymphoproliferative syndrome, and hepatosplenomegaly. The authors claim that the syrup inhibits active replication of the virus and has pronounced immunomodulatory and antioxidant effects [36]. S. O. Kramarev et al. (2008) conducted clinical studies to study the effectiveness and tolerability of Flavozid® syrup for EBVI in children in the reactivation stage. It has been shown that the drug in the complex therapy of the disease increases the effectiveness of treatment, significantly reducing viremia, and does not cause side effects [37, 38].

V. V. Chopiak et al. (2008) presented the results of a study of the medicinal product PROTEFLAZID® (drops) for the treatment of patients with chronic EBVI in the stage of reactivation on the background of replicative activity of the virus (DNA+). Prolonged (up to two months) use of PROTEFLAZID® drops leads to more rapid regression of clinical manifestations of the disease and improvement of the general condition of patients. Specific molecular genetic studies using the PCR method confirmed that the drug has pronounced antiviral properties. Based on comprehensive immunological studies, it was concluded that PROTEFLAZID® contributed to an increase in the number of T cytotoxic lymphocytes (CD8+), stabilization of the functional activity of NK cells, a decrease in the activity of the suppressive subpopulation of regulatory CD4+/CD25+ lymphocytes, and an increase in the absorption capacity of phagocytes. The complex of these factors enhances the antiviral response of the body [39].

In the studies of S. O. Kramareva, O. V. Vygovskaya (2011), the authors observed children with EBVI in the reactivation stage. Clinical and laboratory data showed the effectiveness of Flavozid® syrup, included in the complex therapy of the disease [40]. When studying the effectiveness, tolerability, and possible side effects of the medicinal product Flavozid® (syrup) in 2012 in children, in the treatment of EBVI in the activation stage, the authors confirmed the antiviral activity of the drug Flavozid® (syrup) in the clinic. Serological and PCR studies for the presence of EVB recorded a decrease in the detection of anti-EBV IgM VCA (IgM class antibodies to the capsid antigen of the virus) and a decrease in the detection of EBV DNA in saliva and blood. The drug is recognized as safe in the treatment of acute and chronic active EBVI in children [41].

Kramarev S. O. et al. (2014) in a large study showed the effectiveness of Flavozid® syrup in the treatment of infectious mononucleosis of EBV etiology, which was characterized by a pronounced positive dynamics of T-cell and B-cell immunity indicators, which indicated the elimination of a pronounced inflammatory reaction from the organs and systems of the body and the corresponding clinical recovery, the absence of a protracted infectious process, and the absence of a threat of transition to a chronic form of infection. The results of the studies allow us to speak about the high clinical effectiveness of Flavozid® syrup, which is manifested by reliable antiviral and immunocorrective activity in the treatment of EBV-etiological IM in children [42].

G. A. Biletska (2011) based on clinical data showed that the use of PROTEFLAZID® in the complex therapy of children with IM of EBV etiology reduces the duration of the manifestation of the disease and the duration of the children's stay in the hospital, reduces the possibility of relapses [43]. T. P. Borisova, E. M. Tolchennikova (2013, 2014) evaluated the effectiveness of complex antiviral and immunotropic therapy for chronic EBV infection in children with hematuric form of chronic glomerulonephritis (HGN). The results of the studies indicate positive dynamics in the course of HGN and concomitant chronic EBVI, as well as cytokine status after complex antiviral and immunotropic therapy with the inclusion of the drug PROTEFLAZID®. A decrease in the frequency of respiratory morbidity, subfebrile condition, asthenic syndrome, systemic lymphadenopathy, hepatomegaly, intestinal syndrome, lymphocytosis, and elimination of splenomegaly were noted [44, 45].

The results of the preclinical studies are consistent with the data obtained in practical medicine.

The table below presents the main results of 16 clinical studies in chronological order.

In the clinical studies described in the table, in complex therapy and monotherapy of EBVI, the medicinal products PROTEFLAZID® (drops) and Flavozid® (syrup) exhibit antiviral activity, as evidenced by the disappearance of markers of active virus replication from the blood and saliva of patients: anti-EBV IgM VCA, EBV DNA in the blood after the course of treatment. This established fact is confirmed by preclinical data on the specific antiviral activity of the active substance of the PROTEFLAZID® extract against EBV.

PROTEFLAZID® and Flavozid® reduce the average duration of hospital stay by 1.5 times and to the same extent reduce the severity of neuroautoimmune reactions [28]. It should be noted that the symptoms of intoxication, against the background of taking the drugs, became less pronounced and resolved 2.5 times faster, the course of the disease was milder, and fever was half as short; the duration of lymphoproliferative syndrome was significantly reduced, and the frequency of hepatosplenomegaly decreased.

It has been proven that PROTEFLAZID® and Flavozid® induce the synthesis of endogenous alpha- and gamma-interferons (IFN) in clinical conditions. With daily administration (for 6 months), there is no inhibition of alpha- and gamma-IFN activity, which indicates the absence of refractoriness of the immune system to these drugs.

The immunomodulatory activity of PROTEFLAZID® and Flavozid® medicinal products is manifested in increasing the body's resistance to viral infections, restoring immunity indicators. In the case of EBVI reactivation, the effectiveness and safety of repeated courses of therapy with the drugs PROTEFLAZID® and Flavozid® have been proven.

Table. Results of clinical studies on the efficacy and safety of the medicinal products PROTEFLAZID® (drops) and Flavozid® (syrup) in children and adults from 2002 to 2014.

№	Authors, year, source	Medicinal product	Number of patients		Results of use of PROTEFLAZID®/ Flavozid® in clinical practice
			Total	Taking the product	
1	Matyash V.I. et al., 2002 [30]	PROTEFLAZID®	25 adults	25	A stable effect was achieved in restoring the functions of the affected organs and systems: lymph nodes, liver, and spleen; hemogram indicators improved. On the background of treatment, the symptoms of focal brain damage were eliminated within 2-3 weeks, and coordination and memory improved.
2	Nikoforova T.O. et al., 2004 [31]	PROTEFLAZID®	26 adults	14	The increased body temperature and general condition normalized in 76.8% of patients who received the drug, compared with 50.0% of people in the control group (c.g.). A decrease in lymphadenopathy was noted in 35.7% of patients who took the drug, compared with 25.0% in c.g. Bilirubin and aminotransferase activity in patients who took the drug normalized on average by 3.5 ± 0.1 days faster than in patients in c.g. Under the influence of the drug, the relative number of lymphocytes and mononuclear cells in the blood decreased, and the level of IgG in the blood also significantly decreased. After 1 month, only 7.0% of patients who took the drug had symptoms of hepatorenal syndrome and lymphadenopathy, while in the control group, this indicator was 25.0%.
3	Gley A.I. 2005 [32]	PROTEFLAZID®	47 adults	27	A clear positive dynamics has been described, manifested in a rapid decrease in the size of the lymph nodes and spleen, improvement in hemogram parameters (decrease in the number of leukocytes, disappearance or decrease in the number of atypical mononuclear cells), and biochemical parameters (normalization or decrease in transaminase activity). After 2 months, viremia was detected in 48% of patients receiving the drug and in 98% in the control group.
4	Gley A.I. 2008 [33]	PROTEFLAZID®	258 adults	112	A significant reduction in the duration of viremia was observed with antiviral therapy for 2 months, compared with patients who did not receive such treatment.

5	Usachio- va O.V. et al., 2005 [34]	PROTE- FLAZID®	38 children	17	With almost the same average length of hospital stay (11.05 ± 4.6 and 11.3 ± 4.7) in both groups of patients, 35.3% of children who received the drug were discharged with recovery, compared with 19.1% of children in the control group. In 78.5% of children who were prescribed the drug, a gradual decrease in the severity of hepatomegaly was noted, and in 75% of children with cytolytic syndrome, the ALT level decreased dynamically. The medicinal product was well tolerated by all patients, and no side effects were noted.
6	Pana- siuk O.L., 2006 [35]	PROTE- FLAZID®	236 adults	159	There was a regression of somato-neurological symptoms on the 8th day of treatment ± 2.6 ; seroconversion of antiviral antibodies on the 2nd month of treatment; an increase in the number of NK cells by 17%, increase in the functional activity of mononuclear cells by 20%, neutrophils by 10%, and a decrease in the severity of neuroauto-immune reactions by 1.5 times compared to the control group. Inhibition of α - and γ -IFN activity was not observed. The use of the drug during the convalescence period makes it possible to reduce the frequency of repeated clinical and virological relapses (virus "+") by 1.9 times, clinical relapses (virus "-L") by 1.3 times, reduce the severity of the disease and accelerate the achievement of a therapeutic effect during repeated therapy of relapse.
7	Kharchen- ko Yu.P., Shapovalova G.A., 2007 [36]	Flavozid®	60 children	30	The use of the drug allows to reduce the average length of stay in the hospital by 1.5 times, reduce the duration of the fever by half, reduce the severity of infectious intoxication, reduce the duration of manifestations of damage to the lymphatic system, and the frequency of hepatosplenomegaly. The drug inhibits active replication of the virus and has a pronounced immunomodulatory and antioxidant effect.
8	Chernyshova O.Ye. et al., 2007 [46]	Flavozid®	30 children	18	On the background of the treatment, it was possible to stop the acute manifestations of infection in 67% of children and to transfer the recurrent course of chronic EBVI into a latent state in 64%.

9	Kramarev S.O., Vygovska O.V., 2008 [37]	Flavozid®	35 children	35	In the complex therapy of EBV infection, the drug has antiviral activity, which is manifested by the disappearance of markers of active virus replication from the blood: anti-EBV IgM VCA, EBV DNA after the course of treatment. There was a positive involution of clinical symptoms of the disease: decrease of intoxication syndrome, restoration of appetite, decrease of severity of lymphoproliferative syndrome (size of lymph nodes, liver, spleen), and cessation of hematological disorders.
10	Kramarev S.O. et al., 2008 [38]	Flavozid®	55 children	55	Six months after the start of treatment, anti-EBV IgM VCA continued to be detected in 13.3% of children who were positive on admission; EBV DNA was not detected in the blood. No adverse effects were reported with the drug.
11	Chopiak V.V. et al., 2008 [39]	PROTE- FLAZID®	25 children	25	The medicinal product has pronounced antiviral properties, promotes an increase in T-cytotoxic lymphocytes (CD8+), stabilization of the functional activity of NK cells, a decrease in the suppressive activity of the subpopulation of regulatory CD4+/CD25+ lymphocytes, and an increase in the absorption capacity of mononuclear phagocytes in the blood.
12	Kramarev S.O., Vygovska O.V., 2011 [40]	Flavozid®	60 children	60	After the course of treatment, markers of active replication disappear from the blood: anti-EBV IgM VCA and EBV DNA. The severity of the following clinical symptoms is significantly reduced: CNS disorders, fever, acute tonsillitis, systemic lymphadenopathy, hepatomegaly, and hematological disorders.
13	Biletska G.A. et al., 2011 [43]	PROTE- FLAZID®	75 children	30	The drug in the complex therapy of children with IM shortens the duration of the manifestation of the disease and the duration of the children's stay in the hospital and reduces the possibility of relapses by three times.
14	Kramarev S.O., Vygovska O.V., 2012 [41]	Flavozid®	80 children	40	A significant decrease in the detection of anti-EBV IgM VCA and a decrease in the detection of EBV DNA in saliva and blood relative to the control group were recorded. No side effects of the drug were noted throughout the entire observation period.

15	Boriso- va T.P., Tolchen- nikova O.N., 2013 [44]	Flavozid®	54 children	54	Positive dynamics in the course of hematuric form of chronic glomerulonephritis and concomitant chronic EBVI, as well as cytokine status, as well as the elimination of subfebrile temperature, asthenic syndrome, the frequency of systemic lymphadenopathy, hepatomegaly, intestinal syndrome, lymphocytosis were significantly reduced. Signs of chronic EBVI after treatment are eliminated in 31.5% of patients, compared with the control group.
16	Krana- rev S.O. et al., 2014 [42]	Flavozid®	243 children	60	Indicators of T-cell and B-cell immunity had positive dynamics, which indicated the elimination of a pronounced inflammatory reaction from the organs and systems of the body and corresponded to clinical recovery, the absence of a protracted infectious process, and the absence of a threat of transition to a chronic form of infection.

Conclusions

Preclinical and clinical studies performed in various research institutes and clinics of Ukraine have proven the presence in the active substance PROTEFLAZID® extract and the substance-based medicinal products: PROTEFLAZID® (drops) and Flavozid® (syrup) of the direct antiviral, immunotropic, apoptosis-modulating and antioxidant mechanisms of action. The indicated pharmacodynamic properties of the drugs determine the feasibility of their indication for effective etiopathogenetic treatment of EBVI in children and adults.

The presented studies concerning the drug therapy of EBV, especially in childhood, with antiviral substances of natural origin are of great importance for clinical practice.

Thus, a systematic review of clinical studies performed between 2002 and 2014, involving 1,347 patients (592 adults and 755 children), confirms the effectiveness and high safety profile of the antiviral medicinal products PROTEFLAZID® and Flavozid® for the treatment of diseases caused by the Epstein-Barr virus (cases of mono- or mixed infection) in children and adults. In all 16 clinical studies included in the analysis, positive dynamics during the course of the disease were established.

Analysis of preclinical and clinical research results provides grounds to recommend the medicinal products PROTEFLAZID® (drops) and Flavozid® (syrup) for wide use in clinical practice in the treatment of diseases caused by Epstein-Barr viruses in children and adults.

References:

1. Cohen J. I. Epstein-Barr virus infection. N.Engl. J. Med., 2000 (343). – P. 81.
2. Zhang X. N. et al. Immune evasion strategies of human gamma-herpesviruses: спроможності для віральних тумorigenesis. J Med Virol., 2012 (2). – P. 72.
3. Principles and practice of pediatric infectious diseases. Churchill Livingstone Inc., 1997. – P. 1821.
4. Young L. S. Epstein-Barr virus: 40 years on. Nat. Rev. Cancer., 2004 (10). – P. 757.

5. CDC: Epstein-Barr virus and infectious mononucleosis. (<http://www.cdc.gov/epstein-barr/about-ebv.html>).
6. Papesch M., Watkins R. Epstein-Barr virus infectious mononucleosis. Clin Otolaryngol Allied Sci., 2001 (1). – P. 3.
7. Bennett N. J., Domachowske J. Mononucleosis and Epstein-Barr virus infection. Omaha, NE: eMedicine; 2006. [Accessed 2007 Mar 26]. Available from: <http://www.emedicine.com/PED/topic705.htm>.
8. Kimura H. et al. Prognostic factors for chronic active Epstein-Barr virus infection. J Infect Dis, 2003 (4). – P. 527.
9. Cohen J. I. et al. Епштейн-Барр virus-пов'язаний з лімфопроліферативним захворюванням не безімунокомпromісних hosts. Ann Oncol., 2009 (9). – P. 1472.
10. Kawa K. Epstein-Barr virus-associated diseases in humans. Inf. J. Hematol, 2000 (71). – P. 108.
11. Kawaguchi H. et al. Epstein-Barr virus-infected T lymphocytes в Epstein-Barr virus поєднаний hemophagocytic syndrome. J. Clin. Invest., 1993 (92). – P. 44.
12. Glenda C. Faulkner et al. У небезпечних мононуклеозісах і outs of EBV infection. Trends in Microbiology, 2000 (8). – P. 185.
13. Resting B cells як transfer vehicle для Epstein-Barr virus infection of epithelial cells. Microbiology, 2006 (19). – P. 7201.
14. Jabs W. J. et al.. The primary and memory immune response to Epstein-Barr virus infection *in vitro* характеризується divergent production IL-1beta/IL-6 і IL-10. Scand J Immunol., 2000 (52). – P. 304.
15. Guerreiro M. et al. Human peripheral blood and bone marrow EBV-specific T-cell repertoire in latent infection reveals distinct memory T-cell subsets. Eur. J. Immun., 2010(15). – P. 1566.
16. Kimura H. et al. Prognostic factors for chronic active Epstein-Barr virus infection. J Infect Dis., 2003 (4). – P. 527.
17. Sugaya N. et al. Quantitative analysis Epstein-Barr virus (EBV)-specific CD8+ T cells in patients with chronic active EBV infection. J Infect Dis., 2004 (5). – P. 985.
18. Ahsan N. et al. Epstein-Barr Virus Transforming Protein LMP1 Грати в Critical Role in Virus. Journal of Virology, 2005 (7). – P. 4415.
19. Kalla M. et al. AP-1 homolog BZLF1 з Epstein-Barr virus має дві essential functions dependent on epigenetic state of, viral genome. PNAS, 2010 (107). – P. 850.
20. Bieging K. T. et al. Epstein-Barr virus LMP2A bypasses p53 inactivation in a MYC Model of lymphomagenesis. Proc. Natl. Acad. SCI. USA, 2009 (7). – P. 945.
21. Cohen J. I. et al. Characterization and treatment of chronic active Epstein-Barr virus disease: a 28-year experience в США. Blood, 2011 (22). – P.359.
22. Okano M. et al. Advanced therapeutic and prophylactic strategies for Epstein-Barr virus Вплив на імуніокомпromісний. Експерт. Rev. Anti. Infect. Ther., 2007 (3). – P. 403.
23. Meerbach A. et al. Inhibitory effects of novel nucleoside and nucleotide analogues on Epstein-Barr virus replication. Antivir. Chem. Chemother., 1998 (3). – P. 275.
24. Звіт про науково-дослідну роботу проведення додаткових доклінічних випробовувань нових форм препарату ПРОТЕФЛАЗІД® (Неофлазід). (Вивчення специфічної дії сиропної форми Протефлазиду на віруси Епштейна-Барр та ВІЛ-інфекції). – К., 2003.
25. Старосила Д. Б. Властивості нових сполук з рослинних флавоноїдів та механізми їх антивірусної дії. Київ, 2014. – С. 24.
26. Рибалко С. Л. Звіт про вивчення механізмів дії біологічно активних речовин лікувальної субстанції ПРОТЕФЛАЗІД®. Київ, 2010.
27. Загородня С. Д. та співавт. Дія Протефлазиду на вірус Епштейна-Барр. Мікробіол жур., 2009(1) – С. 57.

28. Крамарьов С. О., Виговська О. В., Тарадій Н. М. Стан апоптозу при гострій Епштейна-Барр вірусній інфекції у дітей. Здоров'я дитини. – 2013. – №7 (50). – С. 151–156.
29. Доклінічні дослідження лікарських засобів. Київ, 2001. – С. 371.
30. Матяш В. І. та співавт. Етіопатогенетична терапія важких форм герпетичної інфекції. Харків, 2002. – С. 28.
31. Нікіфорова Т. О. та співавт. Клініко-імунологічна ефективність протекфлазиду у хворих на Епштейна-Барр вірусну інфекцію. Тернопіль, 2004. – С. 158.
32. Глей А. І. Протекфлазид® у лікуванні хворих на інфекційний мононуклеоз, спричинений вірусом Епштейна-Барр. Суч. Інфекції, 2005 (3–4). – С. 121.
33. Глей А. І. Інфекційний мононуклеоз, етіологічні та клінічні особливості. – К., 2008.
34. Усачова О. В. та співавт. Досвід використання препарату Протекфлазид при інфекційному мононуклеозі у дітей / Репродук. здоров. жінок, 2005 (4). – С. 192.
35. Панасюк О. Л. Етіопатогенетична терапія герпесвірусної інфекції із застосуванням Протекфлазиду та ультрафіолетового опромінення крові. К., 2007.
36. Харченко Ю. П., Шаповалова Г. А. Застосування препарату Флавозид при інфекційному мононуклеозі у дітей // Сучасна педіатрія, 2007 (4). – С. 115.
37. Крамарьов С. О., Виговська О. В. Хронічні форми Епштейна-Барр вірусної інфекції у дітей: сучасні підходи до діагностики та лікування. // Сучасна педіатрія, 2008 (19). – З. 103.
38. Крамарьов С. О. та співавт. Використання препарату «Флавозид» у комплексному лікуванні хронічної активної Епштейна-Барр вірусної інфекції у дітей. // Сучасна педіатрія, 2008 (3) – С. 111.
39. Чоп'як В. В. та співавт. Ефективність монотерапії препаратом протекфлазид при лікуванні хворих на хронічну EBV-вірусну інфекцію у стадії реактивації // Імунол. та алерг., 2008(1). – С. 193.
40. Крамарьов С. О., Виговська О. В. Досвід використання препарату Флавозид при Епштейна-Барр вірусній інфекції у дітей. Сучасна педіатрія, 2011 (5). – С. 16.
41. Крамарьов С. О., Виговська О. В. Звіт про проведення клінічного дослідження ефективності та переносимості препарату Флавозид у дітей з герпесвірусними інфекціями. Клін. імунол. алергол. інфектол., 2012 (4). – С. 18.
42. Крамарьов С. О. та співавт. Лікування інфекційного мононуклеозу у дітей. Сучасна педіатрія, 2014 (3). – С. 18.
43. Білецька Г. А. та співавт. Можливі шляхи корекції терапії інфекційного мононуклеозу у дітей. Тернопіль, 2011.
44. Борисова Т. П., Толченнікова Є. М. Оцінка ефективності протівірусної та імунотропної терапії у дітей з гематуричною формою хронічного гломерулонефриту та хронічною Епштейна-Барр вірусною інфекцією. Здоров'я дитини, 2013 (46). – С. 6.
45. Борисова Т. П., Толченнікова Є. М. Хронічний гломерулонефрит (гематурична форма) та супутня хронічна Епштейн-Барр вірусна інфекція у дітей: особливості перебігу та терапії. Архів клін. експ. медицини, 2014(1). – С. 45.
46. Чернишова О. Є. та співавт. Характер змін здоров'я дітей раннього віку, стан їх імунного та цитокінового статусу на тлі різної течії інфекції, викликаной вірусом Епштейна-Барр. Лікарська практика, 2007 (1). – С.24.

Reference: Grynevych O., Kramarev S., Matyash V., Solomacha L., Vygovskaya O. PROTEFLAZID®: Specific activity in Epstein-Barr virus infection in a preclinical study; efficacy and safety in the clinic (systematic review) /Japanese Educational and Scientific Review. – 2015. – V. No.1. (9). – P. 113–126.

2.5. THERAPEUTIC EFFICACY AND TOLERABILITY PROFILES OF PROTEFLAZID®, SUPPOSITORY AND DROPS AMONG THE PATIENTS WITH HERPESVIRUS INFECTION IN THE EXACERBATION PHASE

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Abstract

Reporting of Phase-II clinical trials results for PROTEFLAZID® (ethanolic extract of *Deschampsia caespitosa* L. and *Calamagrostis epigeios* L.) in the new drug formulation – suppository, among the patients with herpesvirus infection. It had been drawn conclusion about high efficacy PROTEFLAZID® suppositories (Scientific & Manufacturing Company «Ecopharm», Ltd, Ukraine) in the treatment of infections caused by the herpes simplex virus (type 1 and 2) with good tolerability.

Key words: *Deschampsia caespitosa*; *Calamagrostis epigeios*; PROTEFLAZID®, suppository; PROTEFLAZID®, drops; flavonoids; herpetic infection; Herpes genitalis.

Introduction

Relevance. In obstetrics, gynecology, and urology, diseases caused by the herpes simplex virus (HSV) are some of the most important problems. According to the WHO, about 90% of the world's population is a carrier of one or more types of herpes viruses [1].

There is no mandatory registration of diseases caused by HSV in Ukraine. Officially, the incidence of genital herpes in Ukraine is an average of 15 cases per 100 thousand population per year [2]. However, literature data indicate that 10% of women and 15% of men regularly suffer from genital herpes, and the number of carriers is not recorded [2].

In Western European countries, seroepidemiological studies have shown a significant difference between the prevalence of seropositivity and the actual incidence of genital herpes, which exceeds 80 cases per 100 100,000 population, and in the USA it approaches 200 cases per 100 100,000 inhabitants [3].

The generally accepted tactics for treating various forms of herpes infection are regulated by the standards for dermatovenereological care, which are carried out by the Order of the Ministry of Health of Ukraine No. 312 of 08.05.2009 and the Order of the Ministry of Health of Ukraine No. 286 of 07.06.2004. According to these orders, the treatment program used for herpesvirus infection includes taking the drug acyclovir in standard dosages. However, despite the treatment tactics, relapses of the disease are often observed, especially during pregnancy.

To prevent relapse of the disease, various means of stimulating the immune system are currently used, but a stable clinical effect is not achieved in all patients. One of the drugs with proven

immunotropic, antiviral, and antirelapse properties intended for this purpose is PROTEFLAZID® in the form of suppositories [4].

PROTEFLAZID® is an active antiviral drug, which active substance (flavonoids) inhibits the synthesis of DNA and RNA of viruses in infected cells by inhibiting the activity of virus-specific RNA and DNA polymerases, thymidine kinase [5] and reverse transcriptase [6]. The drug promotes the synthesis of endogenous alpha and gamma interferons to a physiologically active level (without the phenomenon of refractoriness), which increases local nonspecific resistance to viral and bacterial infections [7].

Clinical studies have shown that PROTEFLAZID® suppositories restore the protective function of the vaginal and cervical mucosa due to the normalization of local immunity factors (secretory IgA – sIgA, lysozyme, and C₃-component of complement) [8, 9, 10].

It has been established that the active substance of the drug has specific antiviral activity and inhibits HPV reproduction in experimental models of oncogenic HPV strains in vitro. Cytological studies have shown inhibition of the proliferative and destructive effects of HPV on cells under the influence of the drug [11].

In genital herpes, the use of PROTEFLAZID® suppositories prevents the appearance of new elements of the rash, reduces the likelihood of dissemination and visceral complications, accelerates the healing of damaged areas; in case of vaginosis, vaginitis and inflammatory diseases of the cervix – promotes the restoration of local immunity and more rapid and effective elimination of the pathogen. PROTEFLAZID® in the form of suppositories has antioxidant activity, and inhibits the course of free radical processes, thereby preventing the accumulation of lipid peroxidation products, and enhancing the antioxidant status of cells [12]. The drug is an apoptosis modulator, enhancing the effect of apoptosis-inducing factors [13]; namely, by activating caspase-9, it stimulates the elimination of virus-infected cells [14], thereby contributing to the primary prevention of chronic diseases on the background of latent viral infections [15].

In the course of phase I clinical study of the medicinal product PROTEFLAZID® in the form of suppositories, the efficacy, and tolerability of the drug was assessed in 30 patients with genital herpes in the remission phase. Against the background of treatment, a decrease in the level of IgG virus, sIgA, and an increase in the levels of lysozyme and C₃-component of complement in the cervical mucus of patients were noted, which indicated an increase in both the general and local levels of the body's immunological resistance. The drug was well tolerated, and the patients did not experience serious side effects or negative changes in laboratory parameters [16].

Objective of the study: to evaluate the efficacy and tolerability of the medicinal product PROTEFLAZID® (suppositories) in patients with exacerbation of herpetic infection.

Study object and methods. The study is a phase II clinical trial of the medicinal product PROTEFLAZID® in the form of suppositories. The design is an open, controlled, randomized study with parallel groups. The study involved 70 patients aged 18–50 years with a verified diagnosis of genital herpes (HSV-1, HSV-2) in the acute stage, who were undergoing inpatient treatment in the Department of Rehabilitation of Female Reproductive Function of the State Enterprise “Institute of Pediatrics, Obstetrics and Gynecology of the National Academy of Medical Sciences of Ukraine” (Kyiv).

Patients, based on the simple randomization method, were divided into the main (n=35) and control (n=35) groups. All subjects received the drug Herpevir as basic therapy, tablets of 200 mg, 1 tablet 5 times a day for 5 days. In addition, patients in the main group were prescribed the study drug PROTEFLAZID®, suppositories, 1 suppository 1 time a day for 10 days.

Patients in the control group were prescribed the reference product PROTEFLAZID® (drops) in the form of vaginal tampons with a solution of the drug. To prepare the solution, 3.0 ml (72–75 drops) of the drug were diluted in 20 ml of sterile saline solution of sodium chloride. The exposure time of vaginal tampons was 30–40 minutes; the procedures were to be performed 2 times a day for 10 days.

The control of indicators was carried out before the beginning of the study, after the end of the therapy course (10 days) and after the end of treatment (after 8 weeks). The effectiveness of treatment was assessed by the severity of clinical signs of herpetic infection before the end of the course of treatment (after 10 days from the start of treatment), as well as 2 and 8 weeks after the end of the course of treatment. The severity of clinical signs of herpetic infection was assessed based on the subjective complaints of the subjects (itching, burning, soreness) on a 4-point scale (from 0 to 3).

When examining the affected area of the mucous membrane, the nature of the changes in the vaginal mucosa was described, and the severity of inflammatory changes was also assessed (in points). The severity of signs of inflammation was evaluated using the following scale: 0 – absence; 1 – mild; 2 – moderate; 3 – severe degree. The severity of morphological elements of the rash was also assessed using the scale: 0 – absence of signs; 1 – weak degree of severity; 2 – moderate degree of severity; 3 – significant degree of severity. The drug was considered effective if the severity of clinical signs of herpetic infection by the end of the treatment course (10 days after the start of treatment) was slightly more than 1 point (0 – 1 point).

The conclusion about the effectiveness was also made based on the difference in the levels of markers of HSV-1, HSV-2 (IgG, IgM), levels of HSV DNA in smears-scrapes from the epithelium of the vaginal mucosa/cervix, and indicators of local immunity (sIgA, lysozyme, C₃-component of complement). In addition, the frequency of relapses, duration of relapses, and severity of relapses during the observation period were compared. In order to assess the state of the body for possible side effects or reactions, the data of the objective examination (heart rate, blood pressure, body temperature, auscultation of the heart and lungs, data of the examination of the skin and mucous membranes); and indicators of general and biochemical blood and urine tests were taken into account and compared.

Results and discussion. At the screening stage, the normality of the distribution of the subjects in the main and control groups was assessed according to the mentioned indicators. Thus, the indicator “Average age” was 31.1 years and 33.2 years in the main and control groups, respectively. According to the duration of the disease, subjects with disease duration of 1 to 5 years prevailed – 16 out of 35 patients in the main group and 18 out of 35 in the control group. The groups were also compared according to the indicators: “Frequency of recurrence of herpetic infection”, “Gynecological history data”, “Frequency of concomitant diseases” and “Degree of vaginal cleanliness”.

70 women included in the study received a course of therapy for 10 days. After the end of the treatment course and 8 weeks after its completion, HSV DNA was again detected in swabs-scrapes from the epithelium of the vaginal/cervical mucosa, as well as the determination of HSV markers (IgG, IgM).

HSV DNA was detected in 100% of the subjects in each group during the screening, which was the criterion for selecting subjects for participation in the study. After the end of the 10-day treatment course, chlamydial DNA was not detected in each of the groups. The dynamics of the level of HSV DNA viral load in the groups are presented in Fig. 1.

Data on the evaluation of IgG, and IgM HSV in dynamics in the groups are shown in Fig. 2 and 3.

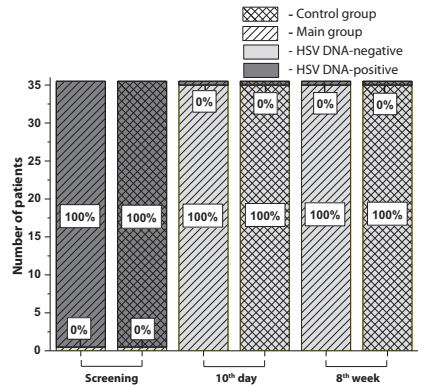


Fig. 1. Detection of HSV DNA in dynamics after taking PROTEFLAZID® suppositories (main group, n=35) and PROTEFLAZID® drops (control group, n=35)

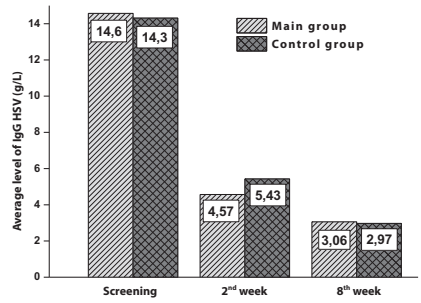


Fig. 2. Dynamics of IgG index (g/l) after taking PROTEFLAZID®, suppositories (main group, n=35) and PROTEFLAZID®, drops (control group, n=35)

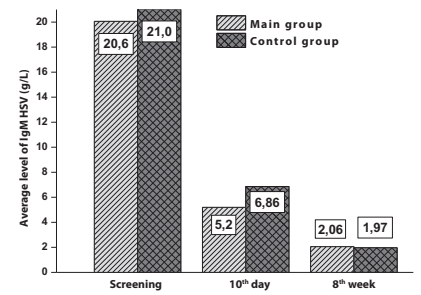


Fig. 3. Dynamics of the "IgM" indicator (g/L) after taking the medicinal products PROTEFLAZID®, suppositories (main group, n=35) and PROTEFLAZID®, drops (control group, n=35)

The data presented allow noting a significant decrease in the level of serological markers IgM HSV and IgG HSV in both groups. After the end of the treatment course, and 8 weeks after the end of the course of treatment, local immunity indicators (sIgA, lysozyme, C₃-component of complement) were re-determined. The data on the assessment of local immunity indicators in dynamics are shown in Fig. 4-6.

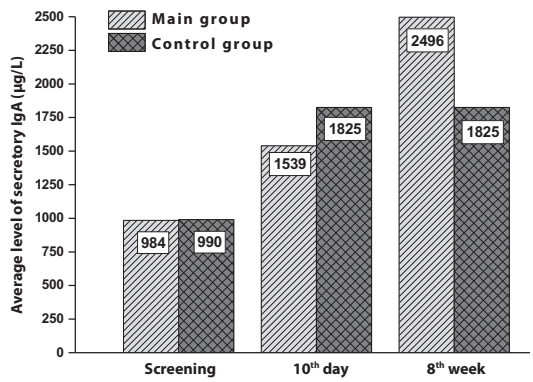


Fig. 4. Dynamics of the indicator “Secretory IgA” (µg/L) after taking the medicinal products PROTEFLAZID®, suppositories (main group, n=35) and PROTEFLAZID®, drops (control group, n=35)

In both groups, significant increases in sIgA and complement C₃ were observed at weeks 2 and 8 compared with screening (Fig. 4-5).

Complement C₃ levels increased significantly by the end of treatment in both groups and returned to baseline by the end of follow-up (Fig. 6).

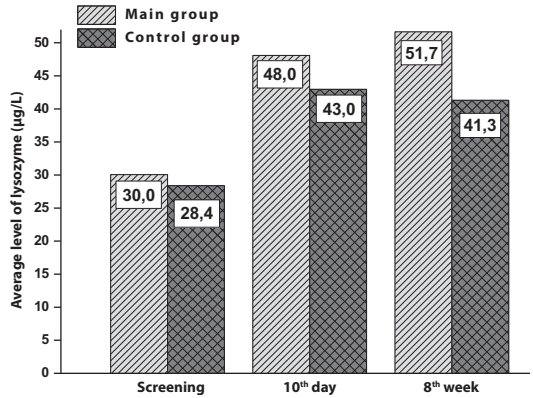


Fig. 5. Dynamics of the indicator “Lysozyme” (µg/L) after taking the medicinal products PROTEFLAZID®, suppositories (main group, n=35) and PROTEFLAZID®, drops (control group, n=35)

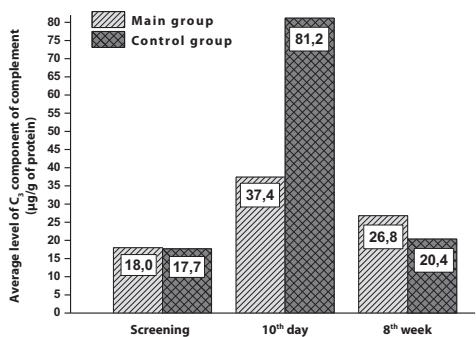


Fig. 6. Dynamics of the indicator “C₃-component of complement” (µg/g protein) after taking the medicinal products PROTEFLAZID[®], suppositories (main group, n=35) and PROTEFLAZID[®] drops (control group, n=35)

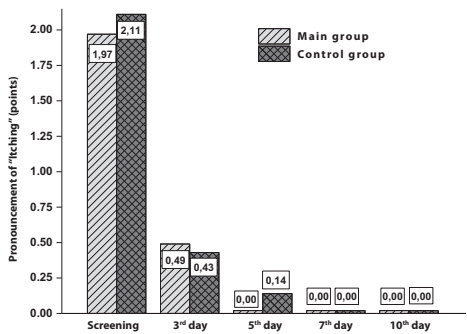


Fig. 7. Dynamics of the severity of the clinical symptom “Itching” (on a subjective scale from 0 to 3 points) after taking the medicinal products PROTEFLAZID[®], suppositories (main group, n=35), and PROTEFLAZID[®] drops (control group, n=35)

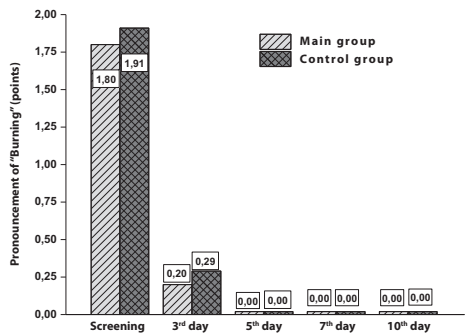


Fig. 8. Dynamics of the severity of the clinical symptom “Burning” (on a subjective scale from 0 to 3 points) after taking the medicinal products PROTEFLAZID[®], suppositories (main group, n=35), and PROTEFLAZID[®] drops (control group, n=35)

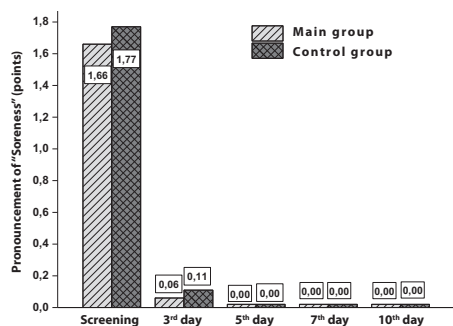


Fig. 9. Dynamics of the severity of the clinical symptom "Soreness" (on a subjective scale from 0 to 3 points) after taking the medicinal products PROTEFLAZID®, suppositories (main group, n=35) and PROTEFLAZID®, drops (control group, n=35)

At each visit following the screening visit, the severity of the subjective complaints (itching, burning, soreness) was assessed on a 4-point scale (from 0 to 3). The dynamics of the assessment of subjective complaints of patients in the main and control groups are presented in Fig. 7–9. The severity of subjective complaints significantly decreased by the third day of treatment, and subsequently, the complaints completely disappeared in both groups. According to the statistical analysis of the registered subjective complaint, the high therapeutic efficacy of the tested drug was noted. In addition, based on the statistical analysis of the recorded laboratory indicators, a conclusion was made about a significant decrease in the level of HSV DNA viral load and a significant improvement in local immunity in both groups.

Instrumental studies of the vaginal mucosa and cervix showed that the drug did not have a negative irritating effect on their condition. Throughout the entire course of treatment and observation, a possible recurrence of herpetic infection was detected and assessed. Over a period of 8 weeks, no recurrences of herpes infection were recorded. In the main and control groups, a significant improvement in colposcopy indicators was noted.

From the data presented in Table 1, it was concluded that the groups did not differ statistically significantly in the effectiveness of treatment. The treatment was found to be equally effective in 35 patients in the main and 35 in the control groups.

Table 1. Patients' distribution by the categories of efficiency parameter

Efficiency	Main group n = 35		Control group n = 35	
	Frequency	%	Frequency	%
Drug is efficient	35	100.0	35	100.0
Drug is non-efficient	0	0	0	0

The conclusion about the unyielding effectiveness of the medicinal product PROTEFLAZID® (suppositories), when compared with the reference product PROTEFLAZID® (drops) in the category of considered patients was made based on the concept of confidence intervals (see Table 2).

Table 2. Limits of 95% CI for the difference in ratios of positive results

Statistical parameter	Value
Probability of error of first kind (α)	0.025
Percentage point of standard normal distribution for α	1.96
Range of effect (δ), %	-20.0%
Fraction of positive results for main group (%)	100.0
Size of main group	35
Fraction of positive results for control group (%)	100.0
Size of control group	35
Difference of fractions, %	0.0
95% Confidence Interval lower limit	-9.89
95% Confidence Interval lower limit	9.89

No serious adverse reactions or side effects were recorded during the study. There were no premature withdrawals of the patients due to an adverse event. The compared drugs had no negative effect on blood pressure, heart rate, and body temperature. After the completion of the clinical study, no negative changes in these parameters were noted in patients of both groups, compared with the baseline level before treatment. Laboratory parameters also did not show any negative changes in any of the cases. No case of exacerbation of chronic diseases was noted.

There were no anaphylactic reactions, delayed-type reactions, or clinically significant fluctuations in hemodynamic and basic laboratory parameters. Based on the presented data, an assessment of the tolerability of the study drugs was carried out (Table 3).

Table 3. Results of tolerability assessment of the studied medicinal products

Tolerability	Main group n = 35		Control group n = 35	
	Frequency	Fraction, %	Frequency	Fraction, %
Good	35	100.0	35	100.0
Satisfactory	0	0	0	0
Unsatisfactory	0	0	0	0

According to the results of statistical analysis, it was concluded that in both groups in most cases there were no statistically significant differences in the majority of the analyzed parameters of general and biochemical blood tests, as well as general urine analysis before and after the course of treatment. The tolerability of treatment was always interpreted as good.

Conclusions:

1) A comparative clinical study involving 70 patients showed high efficacy of the study medicinal product PROTEFLAZID® (suppositories) in the treatment of exacerbation of herpes infection.

2) The study medicinal product PROTEFLAZID® (suppositories) is equally effective as the comparator drug PROTEFLAZID® (drops) in the treatment of recurrences of herpes infection.

3) All women participating in the study, by the end of the 10-day course of treatment, observed relief of clinical manifestations of genital herpes.

4) During the 8-week observation period after the end of the treatment course, patients in both test groups did not experience recurrence of herpetic infection.

5) In both test groups, there was a significant increase in the level of local immunity indicators (sIgA, lysozyme, C₃ component of complement) compared to the baseline. In particular, the level of sIgA increased by the 10th day of treatment, remaining significantly high during the 8-week observation period (from 984.32 to 2,496.19 µg/L); lysozyme levels increased by day 10 of treatment, remaining significantly high during the 8-week observation period (from 30.06 to 51.67 µg/L); complement component C₃ levels increased by day 10 of treatment and returned to baseline by the end of the 8-week observation period (from 17.99 µg/g protein at screening to 37.47 µg/g protein on day 10 and 20.37 µg/g protein on week 8).

6) In both test groups, there was a significant decrease in HSV DNA viral load compared to baseline. After the completion of the 10-day course of treatment, as well as after the completion of the 2- and 8-week observation periods, HSV DNA was not detected in any swabs-scrapes from the epithelium of the vaginal/cervical mucosa.

7) In both groups of subjects, there was a significant decrease in the level of HSV markers (IgG, IgM) compared to the baseline after the completion of the 10-day course of treatment, and 2- and 8-week observation periods.

8) Both drugs demonstrated good tolerability, no adverse reactions or side effects were noted. There were also no negative changes in the data of objective and laboratory examination. The tolerability of treatment in all cases was interpreted as good.

9) The medicinal product PROTEFLAZID® (suppositories) provided a more convenient dosing regimen, unlike the comparator drug PROTEFLAZID® (drops), which required time spent on preparing the necessary dilution and making a swab.

10) Based on the data obtained and presented, the medicinal product PROTEFLAZID® (suppositories) can be recommended as a highly effective and safe antiviral agent for the treatment of gynecological diseases caused by herpetic infection.

References:

1. Arvin A., Campadelli-Fiume G., Mocarski E. et al. Human Herpesviruses: Біологія, Therapy, і Immunoprophylaxis / Cambridge University Press. – 2007. – P. 1408.
2. Мавров І. І. та ін. Основи діагностики та лікування в дерматології та венерології. – Харків: Факт. – 2007. – С. 792.
3. Хрянін А. А. Поширеність вірусу простого герпесу у російській популяції: багаторічний моніторинг // Status Praesens. – 2014. – №6 (17). – С. 67–74.
4. ПРОТЕФЛАЗІД® (краплі) виробництва ПАТ «Фітофарм». – Інструкція з медичного застосування на території України станом на 12 березня 2015 р. – С. 6.
5. Рибалко С. Л. Вивчення мутагенних властивостей препарату ПРОТЕФЛАЗІД®. – Київ. – 2002. – С. 9.
6. Пальчиківська Л. Г. та ін. Антивірусні властивості рослинних флавоноїдів – інгібіторів синтезу ДНК та РНК // Biopolymers and Cell. – 2013. – V. 29 №2. – P. 150–156.
7. Рибалко С. Л. Звіт про вивчення інтерферогенної та антивірусної активності препарату рослинного походження. – м. Київ. – 1997 р. – С. 18.

8. Бенюк В. А. Порівняльна оцінка ефективності та переносимості препарату Протефлазид®, супозиторії, виробництва ТОВ «Фармекс Груп» та препарату Протефлазид, краплі, виробництва ПАТ «Фітофарм» у пацієнток із урогенітальною вірусно-бактеріальною інфекцією. – Київ. – 2015. – С. 71.
9. Камінський В. В. Звіт про клінічне дослідження «Порівняльна оцінка ефективності та переносимості препарату Протефлазид®, супозиторії, виробництва ТОВ «Фармекс Груп» та препарату Протефлазид, краплі, виробництва ПАТ «Фітофарм» у пацієнток з дисплазією епітелію шийки матки, зумовленої папіломавірусною інфекцією». – Київ. – 2015. – С. 79.
10. Корнацька А. Г. Звіт про клінічне дослідження «Порівняльна оцінка ефективності та переносимості препарату Протефлазид, супозиторії виробництва ТОВ «Фармекс Груп» та препарату Протефлазид, краплі виробництва ПАТ «Фітофарм» у пацієнток із загостренням герпетичної інфекції». – Київ. – 2015. – С. 81.
11. Рибалко С. Л. «Звіт про доклінічне вивчення препарату Протефлазид на моделі папіломавірусів». – Київ. – 2010. – С. 32.
12. Рибалко С. Л. Звіт про проведення додаткових доклінічних випробувань препарату Протефлазид на моделі вірусу гепатиту С. – Київ – 2004. – С. 54.
13. Kaminsky V., Litus A., Grynevych O. [et all]. PROTEFLAZID®: Efficacy and Safety in Urogenital Herpes Virus Infection (Systematic Review), 2015. – №2 (16). – P. 705–727.
14. Завелевич М. П. та ін. Інтерферогенна та апоптозмодулююча активність препарату Протефлазид // Вісник Вінницького державного Університет. – 2002. – №6 (2). – С. 281–283.
15. Бондарчук О. Б. Протефлазид як засіб профілактики гострої респіраторної вірусної інфекції (ГРВІ) у дітей // Імунологія та алергологія. – 2004. – №1. – С. 44.
16. Корнацька А. Г. Звіт про клінічне дослідження «Відкрите дослідження з вивчення переносимості та попередньої оцінки ефективності препарату Протефлазид, супозиторії виробництва спільне Українсько-Іспанське підприємство «Сперко Україна», м. Вінниця, Україна у пацієнток із генітальним герпесом у фазі ремісії». – 2013. – С. 67.

Reference: Kornatskaya A., Revenko O., Grynevych O., Shepetko M. Therapeutic efficacy and tolerability profiles of Proteflazid®, suppository and drops among the patients with herpesvirus infection in the exacerbation phase.// European Journal of Scientific Research. – 2016. – No. 1 (13). – P. 507–520



Section 3

3.1. PROTEFLAZID®: EFFICACY AND SAFETY IN CLINICAL PRACTICE FOR DISEASES CAUSED BY HUMAN PAPILLOMAVIRUS (SYSTEMATIC REVIEW)

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Abstract

Systematic review is devoted to the analysis of specific activity, safety and efficacy of the medicinal agent PROTEFLAZID® (drops) in conditions of preclinical investigation and at the stage of clinical observation, in case of diseases caused by human papillomavirus. Independent clinical data (more than 1 500 patients participated) is substantiated and reflected in 20 scientific literary sources that could be compared by goals to results of conducted treatment, and confirm antiviral influence of medicinal agent regarding human papillomavirus.

Key words: PROTEFLAZID®, human papillomavirus, specific activity, safety, efficacy.

Introduction

In practice of obstetricians, gynecologists, urologists, dermatologists and sexologists genital organs' diseases caused by human papillomavirus (HPV), are one of the major problems, as the clinical manifestations, diagnosis and tactics of patients with various forms of human papillomavirus infection (PVI) are inconsistent and not standardized, especially due to absence lack of medicines with active substances, which have a direct mechanism of antiviral action of HPV. The current trend in recent years is unreasonable and sometimes violent treatment of women infected with HPV, often leads to long-term complications, disease recurrence, especially during pregnancy [13, 20, 27].

Viral diseases occupy a prominent place in the structure of urogenital infections. PVI can be a cause of clinical and subclinical diseases of the genital organs. Clinical forms include various types of genital warts that affect the penis, vulva, vagina, cervix and anus. PVI often

(in 60% of cases) combined with inter epithelial neoplasia, and some (cancer-causing) HPV types, penetrating into the cervical area, lead to the development of cervical carcinoma [4, 20]. This is especially noticeable in pregnant women, due to the emergence of a large number of cells, which are divided with impaired differentiation and hyperplasia. This causes activation of PVI and carcinogenesis processes.

The mucous membrane of the cervical canal hypertrophies due to an increase in the size of columnar epithelial cells and strengthen them in the processes of mucus. And also, there is a degradation of collagen fibers, creation of new blood vessels and new cells, which are similar in morphology to decidual in the cervical stroma [13, 20, 26, and 27].

Genital PVI is quite common throughout the world. More than 70 species of AAH are human pathogens. Globally, about 630 million people are infected with PVI. Most frequently detected five highly oncogenic HPV genotypes: PVI_16 (3.2%), PVI_18 (1.4%), PVI_52 (0.9%), PVI_31 (0.8%) and PVI_58 (0.7%) [11, 14, 21].

In most patients, the infection is caused by infection during sexual contact with patients.

The incubation period varies from 3 weeks to 9 months, sometimes greatest, and an average of 2 to 3 months. Virus transmission to sexual partners occurs in the 46-67% cases. Natural reservoir of HPV is considered to be the population of men with Bowenoid papulosis with symptomatic form of the disease, as well as men with subclinical PVI patients and penile cancer. The lesions on the skin and head of the penis, in the urethra, bladder, ureters, cervix, perianal region define a wide range of different types of HPV. Since 1996, WHO has decided to consider HPV types 16 and 18 are carcinogenic to humans, HPV types 31 and 33 may be carcinogenic, and a number of other types of conditional carcinogens [5, 14, 15, 20, 21, 27].

There is evidence that about one in three sexually active women has various forms of clinical manifestations of AAH. 95% of cases, HPV is localized in the area of the junction of stratified squamous and columnar epithelium, where there is epithelial dysplasia of the cervix.

During conducting multicenter studies on the prevalence of HPV in adolescents, the persistence of viruses in a year provided only 30% of cases, after 2 years – 9%. In this clinical regression took place in 80% of patients. Regression of cervical squamous intraepithelial lesions of low degree was observed in 90% of teenagers, while in adult women in 50-80% of cases. These figures confirm the picture of the sexual transmission of the virus. Thus, the risk of cervical disease in women at first sexual intercourse before the age of 18 years, five times the risk of women who had sex after 22 years [6, 11, 15, 20, 27].

Risk factors for developing cervical cancer (CC) include cervical disease which exist for a long time (benign and precancerous), and, if left untreated, lead to cancer.

In carcinogenesis other than HPV, involved a number of other cancer-causing agents of various natures. These include: the early onset of sexual activity (15 years), frequent change of partners (more than 5), smoking (more than 10 cigarettes a day), the lack of sexual health of both partners, a history of an ovulatory menstrual cycles before pregnancy, hyperestrogenia, primary infertility, the effect of radiation before and during pregnancy, birth trauma and cervical abortion, sexually transmitted infections (STIs) [13, 15, 20, 27].

According to the literature, 5-year survival rate of patients with cervical cancer in combination with pregnancy in the early stages (I-II stage) – is 68, 2-77% of cases, in advanced (III-IV stage) – 44-55% [20, 26].

Now it is proved that the viral infection can remain latent for several years, and its presence increases the risk of cervical cancer, on average, 300 times [5, 14]. Infection men is also an important factor as a result of their disease and how carriers of these viruses. HPV can cause in men a significant number of cancers, including cancer of the anus, penis, and oropharyngeal cancer. The frequency of anal and oral forms of cancer associated with HPV, increases in the general population, and even faster in immune-compromised patients, such as HIV-infected. Penile HPV is very common among both heterosexual and among homosexuals, regardless of the age range. Other diseases associated with HPV, which is clinically significant, are genital warts and recurrent respiratory warts [7, 14]. Referring least 40 HPV strains belonging to the genus papillomavirus may infect the skin of the head and shaft of the penis. The warts cells that begin to degenerate, more often are highly oncogene strains, such as PVI-16 and PVI-18 and low oncogene PVI-6 and PVI-11 [7, 15].

One of the most important risk factors for PVI is immune disorders. PVI is often found in patients with dysfunction of cellular immunity, and more frequently in HIV infected and those who have been treated with immunosuppressive therapy. Clinical observations and experimental studies indicate the leading role of the immune system in controlling the occurrence, course and outcome of PVI [5, 7, 20, and 26].

PVI can take place in several steps:

- 1) primary infection, when there is a localization of the virus in a limited anatomic region;
- 2) the persistence of the viral genome in episomal form which is accompanied by production of viral particles during the differentiation of epithelial cells (An opportunity for this step, secondary infection);
- 3) oncogene processes as a result of the interaction of viral proteins with regulatory oncogene – cells after integration of viral DNA into their differentiation genom. Without differentiation of cells the virus cannot be replicated. The period between infection and cell basal release of virus from 3 weeks to 3 months [5, 7, 11, 16, 21].

Widespread replication and dissemination of the virus during primary infection counteract various factors of a natural antiviral resistance of the organism, including the ability of cells of stratified squamous epithelium of the mucous membrane of urogenitals to permanent desquamation and regeneration, production of interferons (IFN) that can limit the dissemination of viruses process within the affected area, and also the action of natural killers (NK cells) and macrophages [4, 14].

Since the virus is a non-lytic, inflammatory response to HPV is much less pronounced than in other infections, for example, *C.trachomatis*. In the early stages of infection with HPV is the body, so to speak, an immunologically not aware of the virus, since the virus are released only in the outer epithelial layer, away from the submucosa, that is, the primary place of the immune defense. At the same time, the primary runs PVI innate immune response through activation of Toll-like receptors (TPR) that recognize pathogen-associated membrane proteins, or through activation of natural killer cells. It is believed that the innate immune response is responsible for the rapid clearance of the organism from

antigens – for a few weeks [4]. Chronic PVI also causes activation of the adaptive immune response associated with the presentation of viral antigens of antigen presenting cells (APCs), such as Langerhans cells and dendritic cells. To successfully acquired immune response is necessary from several months to several years, especially oncogene HPV types, especially PVI-16 can suppress both the innate and acquired immune responses through different mechanisms. Since the MOC is localized mainly in the epithelium, it is believed that the immune responses in this case are mucosal. All of these immune parameters you need to consider when choosing an immune modulatory and antiviral therapy in patients with HPV [4, 15].

Diagnosis of typical manifestations of AAH is not difficult, however, is difficult to define them in the early stages. The main method of diagnosis of atypical varieties PVI is a cytological and histological studies (with the identification coilocytic cells in the biopsy), and polymerase chain reaction (PCR) with the definition of the type of virus [4].

In the literature more than 30 years there is a constant debate about the most appropriate treatment of severe dysplasia, in epithelial cancers (SIN_III) and early forms of cervical cancer in young women. The encouraging results of treatment of early forms of tumors with a gradual decrease in the volume of surgical interventions became an occasion for the development of a new direction in gynecological oncology – organo-preserving treatment (EG Novikova, 1998). Given that a malignant tumor in the pre-invasive and invasive micro stages is local removal of the primary tumor and may lead to a cure, is increasingly used sparing surgical treatment [8, 26, 27].

A common treatment of PVI in the presence of clinical manifestations is a surgical method, including using electrocautery, cryoablation, and others. For therapeutic purposes when HPV is also used drugs that cause chemically induced necrosis exophytic manifestations PVI (Solkoderm, Ferezol, Collomak et al.). To prevent recurrence of the disease is currently used recombinant forms of IFN inducer of endogenous IFN, ointments with antiviral drugs. However, it is possible to achieve stable clinical effect, not all patients [4, 15, 27]. To suppress replication of HPV is necessary to use antiviral agents. Representatives of the group of direct antivirals is a drug Proteflazid® (drops) (SMC «Ecopharm», Kyiv, Ukraine) exhibiting pronounced antiviral activity against DNK viruses, which include viruses genus Papillomavirus (family Papovaviridae).

Proteflazid® (drops) – a liquid alcohol extract obtained from wild cereals pickerel Soddy (*Deschampsia caespitosa* L.) and Veinik Ground (*Calamagrostis pidgeonis* L.). The active ingredients of the drug are flavonoids. Flavonoids are a group of biologically active compounds – benzopyrene derivatives, which are based phenylpropane skeleton composed of units $C_6-C_3-C_6$ -carbonaceous with oxygen atom in the heterocyclic ring. Depending on the degree of oxidation and hydroxylation of propane skeleton and position of the phenyl radical flavonoids are divided into several groups: flavones, isoflavones, flavonols, flavonones and flavanonols. Flavonoids are natural phenolic compounds. The specific properties of the drug are determined by the fact that in terms of pharmacological more than one flavonoid is valid under the conditions of the body, and there is the effect of the system of biochemical transformations the presence of highly active radical intermediates [3, 4, 15, 20, and 22].

Objective

To analyze the specific activity of Proteflazid® (drops) at the stage of preclinical study; evaluate the effectiveness and safety in clinical practice with genital diseases caused by HPV.

Materials and methods

We used scientific publications, reports on preclinical and clinical study, systematic analysis.

Results and discussion

Studies have exposed systematic analysis, carried out at the stages of preclinical and clinical study of the drug Proteflazid® (drops) on the bases NIINANU far and the Ministry of Health of Ukraine (SE «Institute of Urology NAMS of Ukraine, «Kharkiv regional nephrotoxicity urological center named after VI Shapoval, National Medical Academy of Postgraduate Education named after PL Shupyk, Chernivtsi, Vinnytsia, Dnipropetrovsk, Zaporizhia, Ivano-Frankivsk, Kyiv, Luhansk, Ternopil, Kharkiv medical universities, etc.) as well as Scientific and Research Institute of Obstetrics and Gynecology, Ministry of Health of the Republic of Uzbekistan, the Ministry of Health of Kazakhstan.

The study of the specific activity of the drug Proteflazid® (drop) in the preclinical studies. The specific activity of the active ingredient of the drug in Proteflazid® infection caused by HPV, the preclinical study stage is set using HPV culture model (SV Rybalko, 2010). HPV-producing cell culture was obtained by transfection of DNA isolated from human patients infected cells, suspension cultures MT_4 cells (suspension culture of human lymphoblastoid cells) and transplanted cells BHK (hamster kidney cells). HPV testing was performed by PCR on the cytopathic effect of the virus and cytological characterization – mitotic index and abnormal mitosis. HPV isolates tested primers for HPV 16, 18, 31, 33, 35, 39, 45, 52, 58, and 67 of 59_{go} genotypes. It was found that when cultured Proteflazid® transplantable HPV cell culture has a cytopathic effect, a characteristic feature of which was the formation of foci of transformation. The in vitro experiment installed capacity Proteflazid® drug to inhibit the reproduction of HPV in cell culture by 2 lg ID50 [1].

Cytological study confirmed that the level of abnormal Proteflazid® under the action of the drug mitoses HPV infected cells was the same as that of the uninfected cells. Proteflazid® significantly reduces the level of mitotic activity and abnormal forms of mitosis, the cells infected with HPV. These results indicate a relatively high proliferative inhibition of HPV and destructive action on the cells to assume the clinical efficacy of the specific effect of the drug on Proteflazid® oncogenic HPV (Rybalko SL, et al., 2011). Proteflazid® also inhibits the synthesis of virus specific enzymes, thymidine kinase, DNA and RNA polymerases and reverse transcriptase in the virus-infected cells, which is particularly important when a viral infection mix [2].

The inhibition of cell transformation involves three mechanisms: inhibition of cell proliferation, induction of cell differentiation, induction of apoptosis. Stimulates apoptosis Proteflazid® therefore can inhibit reproduction of HPV and cell proliferation. Proteflazid® in infected cells is able to destroy the DNA polymerase mRNA of viruses. This mechanism is detected while testing the drug Proteflazid® in model systems: transcription (DNA dependent RNA polymerase bacteriophage T7) and replication (taq DNA polymerase) [1].

Analyzing mentioned above preclinical studies, we can state that Proteflazid® has specific antiviral activity against DNA-containing HPV.

Proteflazid® induces the synthesis of endogenous IFN- α and IFN- γ and that is one of the mechanisms of antiviral action and is confirmed by detected levels of IFN- α expression, RCC, an RNA L. The drug induces the synthesis of IFN to the physiological level; it increases not specific resistance to viral and bacterial infections. In addition, the drug stimulates tissue macrophages (increasing exciting, absorbing and digesting ability of macrophages), which is an important link in no particular protecting the body from infectious agents, the drug can affect both the early and the late immune response when PVI by restoring performance levels local immunity [1].

The study of efficacy and safety of Proteflazid® (drops) in the clinic in diseases caused by PVI.

In clinical practice, drug Proteflazid® (drops) is used since 2001. A systematic review carried out on the basis of the available post marketing comparative clinical studies and observations in the course of treatment various diseases of the sexual sphere, due to HPV up to and including 2015.

A significant part of the literary publications devoted to clinical experience with the drug Proteflazid® (drops) for opportunistic infections, the development of which takes place in conditions of immune deficiency. The median prominence belongs to herpes, cytomegaloviral infection and chlamydia, which today infect, according to the domestic and foreign literature, more than 90% of the female population, both in our country and abroad. However, in recent years, more and more studies are carried out aimed at preventing the development of cancer pathology, due to its rejuvenation, to provide organic preservation in the reproductive part of humanity. That is to say, adequately chosen etiotropic antiviral treatment is quite effective and can reduce the proliferative activity of the tumors, which allows for conserving therapy [8].

In the treatment of PVI Proteflazid® (drops) is administered orally within 3 months, in accordance with the scheme that is shown in table 1.

Table 1. The scheme of Proteflazid® medicine (drops)

Stages of treatment	dose (drops) and the number of intakes per day
1 st week	7 drops 2 times per day
2 nd and 3 rd weeks	15 drops 2 times per day
4 th week	12 drops 2 times per day

Proteflazid® (drops) can also be used for 15-12 drops 2 times a day, starting from the first day of application. At the same time carry out a local therapy with Proteflazid® (drops) used vaginal swabs with a solution of the drug. To prepare the solution needed 3.0 ml (75-72 drops) to dissolve the drug in 20 ml of physiological sodium chloride solution. Time tampons exposure – 40-30 minutes. Local therapy applied twice a day for 2 weeks [10 ,6, and 23].

Given that one of the main etiological factors in the development of cancer of the genital organs is HPV, of particular importance is the availability of antiviral drugs with specific antiviral mechanism against HPV. Thus, the results of study of V.G. Radionova et al in 2002, on monitoring of patients with various manifestations of HPV in the background of Proteflazid® (drops) administration, with the active examination of patients within 6 months showed the relapse of condylomatous manifestations in the next 3 months after therapy in 4 out of 15 patients from control group (26.7%) and 3 out of 24 patients from main group (8.3%). The positive effect of the drug on the natural resistance of the organism has been confirmed by the analysis of leukogram [4].

Thus, 599 patients who were divided into three groups, based on the prevalence of the etiological factors of cervical pathology, GA Vakulenko, EV Kohanevich and AV Godu marketplace in 2003 [8] The drug treatment was administered Proteflazid® (drops) as a topically (in the form of vaginal tampons diluted 4 : 1 with normal saline) and peros scheme: 1 week – 5 drops 3 times a day, 2 -3 weeks 10 drops three times a day, week 4 -by 8 drops 3 times a day for 1.5-2 months.

The reduction of subjective and objective changes was registered in patients as a result of etiotropic treatment. The area of inflammation focus has decreased, signs of inflammation decreased or disappeared in the colpocervicoscopic picture in 96% of patients. In 3 patients, in whom there has been the spread of vaginal vaults, after local administration of Proteflazid® (drops), the process remained only in the I and II zones of exocervix. In one case, colposcopic signs of dysplasia characterized by fields without the polymorphism; while cytology material cervical canal surface evidence of a moderate dysplasia [8].

Repeated bacterioscopic inspection revealed the absence of pathogenic microorganisms in 93.7% of patients. The frequency of HPV DNA detection by PCR decreased s69, 2% (before treatment) to 5.4% (after treatment). The immunological imbalance declined in 74% of patients in this group, but the normalization of all investigated immunological parameters was not achieved [8]. According to the study V.V. Makagonova, N.G. Korniets, N.A. Udovik (2003) with the participation of 48 women with benign cervical pathology in age from 20 to 45, who were hospitalized in the gynecological department of the 2nd maternity hospital of Luhansk, show that in 21 women (43.8% of the total surveyed) according to PCR was diagnosed with PVI and / or herpes infection (GUI). Of the 48 tested, 41 women (85.4%) as a treatment for cervical CO₂ – laser milling has been performed. At the stage of preparation before the operating conducted anti-inflammatory immune modulating therapy. Laser vaporization was performed on 6-7 day of the menstrual cycle [9].

The best results were obtained in the study group patients with HPV and / or GUI, part of which included therapy with Proteflazid® (drops) already on day 21 of treatment in %88.88 of the cases reported disappearance of these viruses replicate in the cells of stratified epithelium. In comparison to the group 21^y day of treatment effect was registered only in %58.34 of patients. SO₂ lazerovaporizatsiya pathological sections in all cases lead to complete epithelialization of the cervix, which is confirmed by the results of colposcopy. Repair processes in all cases 5^y ended at week after surgery. When viewed from the control study group patients, the disease relapses were observed [9]

Analyzing the experience of L.M.Malanchuk, T.V.Zaykova, V.M. Flehnera and NI Bagno obtained in 2003 as a result of examination and treatment of 22 women with diagnostically confirmed cervical dysplasia mild, we conclude that the use of antiviral drugs, IFN- α and IFN- γ inducers, in particular, the modern Proteflazid® (drops), is effective in complex therapy of cervical dysplasia. Simultaneous oral and vaginal inside the drug improves the effectiveness of therapy by %30 than when only orally, which makes it possible to provide effective prevention of secondary cervical cancer [12].

The study of 27 women with PVI on a background of atrophic vaginitis with post-varioectomic syndrome, conducted in 2003 by C.A. Galnykina on the basis of medical diagnostic center (Ternopil), was aimed at studying the effect of Collomak in topical administration, in combination with Proteflazid® (drops) by oral and topical use in combination with hormone replacement therapy on the clinical course of PVI in women post-varioectomic syndrome, a condition of the system of lipid peroxidation and cellular and humoral immunity, given the important role of these disorders in the pathological process. [18]

It was found that before treatment in the body of examined women there were constant changes in the immune regulatory interaction of relations system of cellular and humoral immunity, which cannot be considered adequate. So, there was a lack of cell-mediated immunity, as evidenced by the reduction in the number T-lymphocytes at the cost of T-helpers. The number of T-suppressors/killers (T s/k) was significantly higher of control markers respectively, immunoregulatory index was decreased. Assigned therapy with Proteflazid® (drops) eliminated the identified immuno suppression. That is, by 30th day from the start of treatment, the deficiency of general population of T-lymphocytes was partly eliminated (CD3); there was a clear trend of reduction of T s/k-CD8, the number of which reached the normalization by 10th day; physiological stabilization of the number of T lymphocytes (CD3) and the normalization of the regulatory index, followed by the restoration of an adequate response of humoral immunity – B- lymphocytes (CD19) [18].

Therefore, in women with PVI on a background of atrophic colitis due to postvarioectomic syndrome who have been treated with Proteflazid® (drops) and Collomak (drug for cytodestructive effects on pointed warts of vulva and vagina), decreased antigen excess tension and immune deficiency states, improved processes antioxidant system, decreased lipid peroxidation, which contributed to the normalization of the pathogenetic mechanisms of disturbed PVI, which indicates a high clinical efficacy of prescribed therapy [18].

In studies by I.T. Kishakevich (2003) 60 women with cervical background diseases (from including 28 women who had a viral infection (HSV – 1,2^{so} types, HPV types 6-11^{so}, 16-35^{so} types 18-59^{so} types), 16 women had mixed infection (a combination of a viral infection with chlamydia, mycoplasmosis or ureaplasmosis) were examined; in accordance with genital infections detected, the women were divided into 2 groups: 1st group – women with a viral infection, 2nd group – viral infection in combination with other genital infections. 1st group of women received in the treatment of the antiviral drug – Proteflazid® (drops), 2nd group of women – acrid one acetic acid [19].

In analyzing the data obtained during use prescription drugs showed increased levels of lysozyme in the serum and in cervical content, as compared with the level of lysozyme before treatment which were substantially close to normal levels. It should be noted that lysozyme activity in cervical contents when using Proteflazid® (drops) in women of 1st group was slightly higher than 0.24 ± 12.85 mcg / ml, than in administration of acrid one acetic acid in 2nd group of women 12.54 ± 0 , 18 ug / ml, and content analysis of serum lysozyme showed efficacy greater Proteflazid® drug (drops), which was used in women 1^y group 8.87 ± 0.35 pg / ml, compared with locusts acetic acid, which was used in women 2^y group 8.64 ± 0.12 ug / ml [19]. Lysozyme levels of serum and cervical content can be one of the criteria for diagnosis of background cervical diseases associated with viral infections, which in immune modulating treatment experienced complete recovery of its activity [19].

According to I.T. Kishakevich (2004) in a survey of 120 women, HPV was detected in 45 women (37.5%). In most cases of HPV was diagnosed in bacterial viral associations, which significantly affect the state of the vagina microbiocenosis and contribute to the progression of the background cervical pathology manifesting by pathological changes of cervical epithelium, colposcopic and cytological signs of a viral infection in 30.7% of subjects. It is found that the therapeutic effect Proteflazid® (drops) with pseudo-erosions of the cervix on the background of PVI and GUIs due to clinical and laboratory effects and provides: acceleration of epithelialization of cervical mucosa with normoplasia of flat multilayered epithelium; restoration of vaginal micro-biocenosis, due to the formation of humoral antibodies against pathogenic microorganisms; balance the local immunity, due to induction of IFN; It eliminates viruses, which is confirmed by PCR studies; reduces pathological focus area and suppresses the inflammatory process; improves the reproductive function, reduces the recurrence rate [11, 24].

Drug treatment of domestic pharmaceutical product Proteflazid® (drops) promoted normalization colposcopic pattern in 62.4% of patients. It should be noted that the highest efficiency was low in patients with cervical lesion of uterus, moderately expressed relief and in patients who did not have extra genital diseases [11].

After 8-10 weeks after completion of antiviral treatment of the repeated diagnosis of PVI by PCR in 85.8% of patients were negative survey data [11].

Thus, the simultaneous use of Proteflazid® (drops) into and locally reduces the affected area, and the degree of severity of the process that allow to treat this pathology conservatively in %62.4 of patients [11].

Analyzing the experience of a comprehensive survey of 11 women aged 22-39 years diagnosed with cervical cancer (8 – John situ conservation, 3 – stage Ia1), which were held organ-preserving surgery (amputation and electro cauterizing conization of cervix uteri followed by laser destruction) in the study of cervical pathology Cherkassy Regional Oncology Center by A.V. Paliychuk in 2004, after the combined treatment with antiviral, antimicrobial and immune correcting preparations using the drug Proteflazid® (drop), it is proved that the use of a comprehensive anti-viral and immune therapy, in patients with early forms of cervical cancer with the presence of infection with herpes simplex virus (HSV) and HPV, provides an opportunity to organpreserving surgery. Treatment of deregulatory disorders of immune system functions and dysfunction secondary cellular

immunity arising in the postoperative period may treat immunocorrective immunomodulating drugs, including the inclusion in national drug regimens Proteflazid® (drops) [6].

Study of A.A. Mamon (2004), conducted in patients with skin PVI, disease duration of which exceeds one year, with one or multiple focal poly clinical forms, proves that the method of complex treatment of the individualized using Proteflazid® drug (drops) as compared with the conventional therapy, improves the quality of treatment to prevent and reduce complications in patients receive results that They were resistant to therapy, and significantly reduces the number of recurrences [25].

D.D. Kurbanov and colleagues in 2004 showed that adequately matched etiotropic treatment with Proteflazid® (drops) in combination with an antimicrobial, anti-inflammatory therapy and substances that normalize vaginal biocenosis is very effective is to reduce the proliferative activity of the tumors, which allows for conserving therapy.

They have also proven not only beneficial effect of the drug on the infectious component in pregnant women, but also its complex effect on the course of the pregnancy itself. That is, the use of Proteflazid® (drops) to reduce the frequency of threatened abortion from %73.3 to %36.7, placental insufficiency from %46.7 to %23.3, pre-eclampsia varying severity from 23.3 to 10% , 0%, premature birth from 16.7% to 6.7%, premature rupture of the membranes from 46.7% to 20.0% and anomalies of labor activity from 26.7% to 10.0%. In addition to the positive aspects of the intake of this drug also provides a reduction of newborn asphyxia of varying degrees of severity – from 43.3% to 23.3%, intra amnionic manifestations of infection – from 23.3% to 10.0% [26].

Building on the work of the Kharkiv scientists OV Grishchenko VV. Bobritskaya, SA Pak (2005), it can be argued that Proteflazid® (drops) is the drug of choice for etiopathogenic therapy in pregnant women, due to lack of teratogenic effects of the drug, and its ability to inhibit the replication of viral DNA. Due to the use of the drug Proteflazid® (drops) in patients of the main group was obtained regression of genital warts in 5-7 days after the start of treatment, complete disappearance of the warts was observed in 76.2% of patients, the remaining 23.8% of women expressed regression of warts in reducing the size of formations on the skin and mucous membranes. Relapse or progression of the process was not observed in the study group patients, the effectiveness of the drug Proteflazid® (drops) was %100. Patients in the control group who received topical treatment drug Collomak without a systemic immune modulation therapy, 41.7% patients had recurrences of external manifestations of the disease in the form of re-education warts or the appearance of new lesions as small warts. [10]

This therapy was used in 21 patients in gestation from 30 to 41 weeks, who received systemic antiviral treatment with Proteflazid® (drops) according to scheme recommended by the manufacturer for 5 drops 3 times a day for two days, 8 drops 3 times a day for two days, then 10 drops three times a day in months techenie3-2 during repeated cycles of pregnancy and after childbirth (minimum rate – 1.5 months).

The duration of dosing up to 3 months was due to the prevention of recurrence of the disease and the achievement of sustainable clinical effect. Condylomata were treated with Collomak and Proteflazid® solution in the form of applications (lotions) [10].

Clinical study of V.V. Bodyan (2005), which involved 45 women with cervical warts of vagina and vulva, infected with HPV treated by Proteflazid® (drops) per os and locally confirms that this therapy provides a significant reduction in the area of lesions on the vulva, vagina and cervix [22].

Analysis of the clinical trial by V.M. Lesovoy and A.V. Yakovleva (2006), Kharkiv regional nephrotoxicity urology center, in 34 patients (8 women 126 men) with PVI highly oncogenic strains, 20 of which received Proteflazid® (drops) in combination with specific therapy, suggests that this therapy significantly affects the elimination of pathogens from the body, and the positive effect is more stable in nature [5].

Based on the results of PCR analysis, it can be argued that in patients receiving Proteflazid® (drops), significantly reduced the risk of transmitting an infectious agent sexual partner, as well as the risk of the virus-induced neoplastic processes (by 5%) [50-47].

According to clinical studies conducted by employees of Zaporozhye MAPS (NN Voloshin et al., 2007) on the basis of the Zaporozhye Regional Clinical Oncology Center at 136 pregnant women with cervical pathology associated with PVI, which in the II trimester were treated with Proteflazid® (drops) orally and topically, there was no progression of precancerous lesions to invasive cancer, which was the basis for deferred treatment after completion of the pregnancy [13].

In identifying clinical, and subclinical form of PVI and II CINI abortion is not shown, but it is necessary to conduct dynamic monitoring and antiviral therapy [13].

In 2007 T.V. Gerasimova, A.N. Gopchuk have conducted study involving 50 women with menstrual irregularities (NMC) and HPV who have been treated with Proteflazid® (drop) per os and locally on the background of symptomatic therapy, showed that the clinical efficacy of the proposed method was 82.8% (normalization of the menstrual cycle). The absence of the pathogen in PCR was observed in 32 (64.0%) women. Lymphocytic link systemic immunity is informative with respect to the forecast of possible systemic disorders. The results of studying the effect of the proposed method on the humoral immunity (the dynamics of changes in the content of the main classes of immunoglobulins (A, F, G) in the serum, as well as normalization of IFN-a, and IFN-y) showed an improvement immunogenesis processes.

The results confirm immunity changes the clinical effectiveness of the proposed method and explain the highlights of changes in the state microbiocenosis genital tract and the immune status of patients with the NMC on the background of a viral infection. The complex of medical preventive measures among women of reproductive age in the background of viral infection, can reduce the frequency of the NMC in 5,3 times, including hypomenstrual syndrome – in 2,2 times, hypoestrogenism – 3.5 times, and restores hormonal balance, immune status microbiocenosis and genital tract. Using antiviral drug immunocorrecting Proteflazid® (drop) in the combined treatment with symptomatic therapy reduced the number of relapses of HPV- 2-1.5 times, in the case of herpes infection – B3-2 times [17].

N.N. Voloshin in 2007 released data that Proteflazid® (drops) in infection with HPV should be used within 6 months against a background of morphological and

cytomorphologic and PCR studies, as well as the fact that the drug is not cause complications, is well tolerated, has a strong antioxidant effect [27].

Analyzing the study conducted in 2010 by I.I. Gorpinchenko and O.V. Romashchenko on the basis of sexual pathology and andrology department of SI «Institute of Urology of NAMS of Ukraine», in the study of 32 women with chronic inflammatory diseases of the pelvic organs with PVI (latent) and disease duration from 2 to 5 years, followed by the appointment of systemic and local treatment of Proteflazid® (drops) showed improvement of general well-being and decrease secretions (intensity) and hyperemia of the genital tract in %59,4 of patients after a week of scheduled therapy and further were combined with the normalization of titles IFN production of tumor necrosis factor (TNF), functional activity of phagocytic cells, cellular and humoral immunity after treatment. The effectiveness of such treatment according to genetic studies using PCR was 81.3%. There has been persistent therapeutic effect colposcopic pattern in 24.9% of women in the form of complete epithelialization of the cervix and the eroded surface of the mosaic epithelialization in 32.2% of patients [15].

E.I. Liliantsy, N.R. Safronnikova, L.A. Redko, V.M. Merabishvili in 2010 at the City Clinical Oncology Center in St. Petersburg conducted a study involving 625 patients with virus types associated pathology.

All patients were divided into two groups: the first group of patients have been treated with Panavir – intravenously and topically, the second – Proteflazid® (drops) per os and locally. The study was a negative result of PCR in 70% of patients receiving the drug Panavir and in 89% of patients who took Proteflazid® (drops) [16].

In 2011, to assess the impact of the drug Proteflazid® (drops) for HPV in males' sexual pathology and andrology department of SI «Institute of Urology of NAMS of Ukraine,» the study was conducted, which was attended by 187 men infected with HPV. M.G. Romanyuk, A.M. Kornienko and P.V. Aksenov demonstrated that exchange the drug reduces the frequency carriers are highly oncogenic HPV strains and improves the results of surgical treatment of penile warts in men, reducing the frequency of relapses in 1.5 times, and the degree of dysplasia tissue in recurrent warts. Perspective is a combination of oral course of Proteflazid® (drops) and the topical application of its solution, which can lead to regression of warts without surgery and reduces the incidence of recurrence compared with only oral administration [7].

On the basis of the Vinnitsa hospital antenatal clinic number 1, since 2012, the employees of the Vinnitsa National Medical University (N.I. Pirogov – N.A. Godlewski and A.V. Starover) were examined 32 women with cervical pathology associated with HPV and treated drug Proteflazid® (drop) as monotherapy with systemic and local his appointment. Due to such treatment the decrease in viral load is set on PCR results for PVL 3,6lg / 105 cells, the normalization of the cervix uteri of women in 68.8% and 31.2% in a significant improvement, i.e. Therapeutic efficacy was 100%, which allowed the researchers to avoid or reduce the degradation of the area of the cervix of the affected area, which is especially important for women planning to give birth [14].

In the years 2010-2013 on the basis of Odessa Regional Hospital, V.N. Zaporozhan, V.G. Maricheredoy, L.I. Dimcheva have conducted the study to evaluate the clinical efficacy of flavonoid glycosides derivatives in the treatment of benign and precancerous cervical conditions.

The study involved 80 women of reproductive age with verified epithelial dysplasia of the cervix uteri mild to moderate (main group) and 30 healthy women who were examined in the order of the clinical examination. The main group was randomly divided into two

subgroups depending on the clinical treatments. In the subgroup of I (n = 40) used the standard medical therapy, and in the II subgroup (n = 40) used the direct antiviral drug with immune modulating action Proteflazid® (drops) orally (8-4 weeks) and topical (vaginal swabs) on 14 days, 2 times with an interval of 10 days [28].

The results of the study suggest that the standard drug therapy is inferior to virostatic and antirecurrent effect of treatment with Proteflazid® (drops). In the usual way the treated patients' viral load is practically constant, whereas in group II had reached a significant reduction of HPV 3,0lg D₅₀. At the same time, under the action of the drug Proteflazid® (drop) increases the number of antigen-presenting cells. The results indicate the appropriateness of Proteflazid® (drop) in the complex therapy in all patients with PVI [28].

To evaluate the efficacy and safety of Proteflazid® (drops) in patients with dysplasia of cervical epithelium caused by PVI, in 2015-2014 a study was conducted of Obstetrics, Gynecology and Reproduction of the National Medical Academy of Postgraduate Education named after PL Shupyk on the basis of the Kyiv City Center for Reproductive and Perinatal Medicine under the direction of a member of. corr. NAMS of Ukraine, Doctor of Medical Science, Professor V. Kaminsky. The study involved 76 women with verified diagnosis of dysplasia of cervical epithelium caused by PVI. Patients using the method of simple randomization were assigned to the main (n = 38) and control (n = 38) group. Patients in the control group was administered Proteflazid® (drops) of production of SMC «Ecopharm» in the form of vaginal swab with a solution of the drug (in 3.0 ml of the drug in 20 ml of physiological sodium chloride solution) na30-40 minutes, 2 times a day [29].

According to the results of the study after 14 days course with the use of tampons solution Proteflazid® (drops) and at the end of the observation period of 8 week there was normalization of the cytological picture or change of cervical intraepithelial neoplasia grade CIN_II (moderate dysplasia) to CIN_I (weak degree of dysplasia), reduces the severity of signs of dysplasia of cervical epithelium, there was significant compared with baseline, the decrease in viral load of HPV DNA. This change was especially pronounced after 8_nedel-nogo observation period (from 2.37 to 0.71 points), suggesting a delayed antiviral effect of the drug. Use of the drug eliminates disbiotic state of vaginal micro flora and restores normal biotope of genital tracts [29].

Table 2. The results of clinical studies on Proteflazid® (drops) efficacy and safety in PVI from 2002 to 2015

№	Authors, year, source	Number of patients		The results of using of Proteflazid® (drops) in general clinical practice
		General	Took per orally/ local applications	
1	V.G.Radionov et al., 2002 [4]	39	24 / 0	Observation for 6 months showed that relapse condylomatous manifestations in the next 3 months of taking the drug after drug therapy Proteflazid® came at %8.3, against %26.7 in the control patients group. The positive effect of the drug on the natural resistance of the organism is confirmed by the analysis carried out leukogram.

2	G.A. Vakulenko et al., 2003 [8]	599	533 / 533	Proteflazid® in combination with etiotropic treatment (with antimicrobial, anti-inflammatory therapy and means of normalization of vaginal biocenosis) is very effective and reduces the proliferative activity of cervical tumors that allows you to apply conserving therapy. The detection rate PCR of HPV DNA in cervical cancer patients decreased from 69.2% (before treatment) to 5.4% (after treatment).
3	V.V. Makagonova et al., 2003 [9]	48	9/9	Patients with HPV and / or HSV, in the therapy which included Proteflazid®, already at 21 Day treatment in %88.88 of cases reported the disappearance of replication of these viruses in the cells of stratified epithelium. In comparison group – only %58.34 of the patients. CO ₂ laser vaporization of pathological areas led to a complete epithelialization of the cervix, which is confirmed by the results of colposcopy. Repair processes in all cases were ended on 5 th week after surgery intervention. On examination, the disease relapses were observed.
4	L.M. Malanchuk et al., 2003 [12]	22	22 / 12	In patients who have been treated with Proteflazid® per orally, dysplasia regression occurred in %60 of cases, in a group where, in addition to oral administration, and still used vaginal swabs with drug – in %83.3 of cases, respectively. Therefore, simultaneous oral and vaginal taking the drug increases the effectiveness of treatment by %30.
5	I.T. Kishakevich, 2003 [19]	44	28 / 28	Antiviral therapy with the drug in the treatment of background Proteflazid® cervical disease provides full recovery of lysozyme activity (Factor specific and non-specific immune responses) in the blood serum and the content in cervical.
6	I.T. Kishakevich, 2004 [11]	120	45/45	Proteflazid® contributes to the normalization of clinical, laboratory parameters and elimination of HPV in %85.8 of patients, which was confirmed by PCR.

7	O.V. Paliychuk, 2004 [6]	11	11/0	In patients with early forms of cervical cancer with the presence of the virus HSV infection and HPV use of combination therapy with antiviral, antimicrobial and immune correcting preparations using Proteflazid® preparation allows for organ-preserving surgery. also recommended the inclusion of the drug in the complex troubleshooting of dysregulation disorders of immune system function and dysfunction of secondary cellular immunity arising during the postoperative period.
8	O.S. Galnykina, 2004 [18]	53	27 / 27	Proteflazid® reduces excess tension and antigenic immunodeficiency, improves processes of antioxiode system reduces lipid peroxidation, which contributes to the normalization of the pathogenetic mechanisms of disturbed PVI that It is an important pathogenetic treatment. The product allows you to eliminate the failure of the immune system. It is noted that in the 30 hours from the start of treatment was partly overcome shortages of the general population of T lymphocytes (CD3) – 58,3 ± 2,9%, there was a clear trend towards a decrease in T / s CD8 – 23,1 ± 1,7 %, whose number reaches normalization 10 hours. The physiological stabilization of the number of T lymphocytes (CD3) and the normalization of the regulatory index accompanied by the restoration of an adequate reaction of humoral immune B lymphocytes (CD19) -? 9, 5 ± 1, 0%.
9	O.V. Gerashchenko, 2005 [10]	33	21 / 21	The regression of genital warts in the group where Proteflazid® was administrated was observed after 5.7 days after the start of treatment. Complete relief was observed in 16 of 21 patients, in 5 pregnant regression of warts is expressed in reducing the size of formations on the skin and mucous membranes. Relapses or progression of the process was not observed. Patients in the control group in 5 cases out of 12 observed marked recurrence of external manifestations of the disease in the form of reeducation warts or the appearance of new lesions.

10	V.V. Bodean 2005 [22]	45	45 / 45	After 8, 10 weeks after treatment in 48% of patients with HPV in samples not determined. The tendency to decrease in the area papillomatous lesions genitals.
11	V.N. Lesovoy, E.V. Yakovlev, 2006 [5]	34	20 / 0	The preparation increases the effectiveness of therapy by 15, 17%. Besides, positive effect is more persistent. The risk transmission to sexual partner, as well as the risk of virus-induced neoplastic processes at 47, 50%.

According to the immunoassay by the end of the course of treatment Proteflazid® (drops) showed an increase in the level of local immunity (secretory of IgA – from 1553 to 2234 ug / L; lysozyme – from 26.73 to 45.33 mg / L; the C₃ component of complement – from 15.41 to 40.29 mcg / g protein) [29].

The main results of clinical observations are shown in Table. 2.

Conclusions

A systematic analysis of the available literature devoted to the results of preclinical study, indicates the presence of the active ingredient of the drug Proteflazid® (drops) direct antiviral action against DNA containing HPV. Along with this, Proteflazid® (drops) has the ability to enhance the synthesis of interferon, which plays an important role in the indirect antiviral effect of the drug. A systematic analysis of the available literature, devoted to the results of clinical application of the drug Proteflazid® (drops), confirmed the antiviral activity of the drug against HPV, confirming the results of preclinical phase of study proves the effectiveness of the drug for diseases caused by HPV in women (including in pregnancy) and male reproductive age. High clinical effect obtained by using drug therapy Proteflazid® (drops) in more than 1,500 patients. Safety confirmed by the absence of side effects. Information is based on the results of 20 independent clinical observations coincidence orientation results of the treatment.

Results from clinical studies indicate that Proteflazid® (drops) poly drug has poly-pharmacological action, namely antiviral, immunocorrective, antioxidant and apoptose modulating that contributes to the achievement of lasting therapeutic effect of HPV eradication, elimination dysbiotic status vaginal microflora, restoration of normal biotope of the genital tract, reduce the frequency of relapses and HPV infection, as well as normalization of cervical status, reduction of proliferative activity of tumors that allows for conserving therapy in the reproductive part of the population as a guarantee of future parenthood.

The Proteflazid® (drops) in the local, intravaginal therapy normalizes the cytological picture and promotes the transformation of cervical intraepithelial neoplasia from CIN-II (moderate dysplasia) to CIN-I (low dysplasia), reducing the severity of symptoms of cervical dysplasia epithelium, contributing significant at compared with baseline, a decrease in viral load of HPV DNA.

Proteflazid® (drops) in the treatment of diseases associated with the PVI contributes to the normalization of clinical and laboratory indices and the elimination of HPV more than 3,6lg in %80 of patients, which was confirmed by PCR.

The efficacy of Proteflazid® (drops) at the stage of preconception preparation of infected women to pregnancy and reducing the incidence of perinatal complications gestational period. For a long time the drug was not pregnant adverse reactions were registered.

Given the high efficacy and safety of drug Proteflazid® (drop) in the treatment of PVI, its use is justified and implemented in a number of guidelines and newsletters in Ukraine and abroad [23-34 ,30 ,29 ,26].

References:

1. Доклінічне вивчення препарату Протефлазид® на моделі папіломавірусів [Звіт] / ДУ «Інститут епідеміології та інфекційних захворювань імені Л. В. Громашевського АМНУ». – К., 2010. – С. 33.
2. Рибалко С. Л., Порва Ю. І., Старосила Д. Б., Дядюн С. Т., Руденко А. В. Моделювання папіломавірусної інфекції *in vitro* та вивчення впливу дії Протефлазиду на репродукцію вірусу папіломи // Матеріали симпозиуму (7–9 червня 2011 р.). – СПб. – 2011. – С. 29–31.
3. Трохимчук Т. Ю., Завелевич М. П., Фільченков А. А., Рибалко С. Л., Гриневич О. Й., Іванська Н. В., Атаманюк В. П. Протефлазид®: антиретровірусна активність // Biological science [Materials of the International Scientific and Practical Conference «Prospects of World Science; Sheffield, Science and Education Ltd.»] – 2014. – July 30 August 7. – Volume 7. – P. 30–38.
4. Радіонов В. Г., Шведюк С. В., Семиряд Ю. В., Радіонов Д. В., Шедання І. Є., Коротін Ю. Л. Застосування Протефлазиду в комплексній терапії папіломавірусної інфекції // Український журнал дерматології, венерології, косметології. – 2002. – №4. – С. 86–90.
5. Лісовий В. М., Яковлєва Є. В. Ефективність препарату Протефлазид® у комплексній терапії хворих з урогенітальною папіломавірусною інфекцією // Здоров'я чоловіка. – 2006. – №2. – С. 230–232.
6. Палійчук О. В. Клінічний досвід використання препарату Протефлазид® у комплексному лікуванні хворих з початковими формами раку шийки матки // Здоров'я жінки. – 2004. – №2 (18). – С. 49–50.
7. Романюк М. Г., Корнієнко А. М., Аксьонов П. В. Використання Протефлазиду для лікування папіломавірусної інфекції у чоловіків // Здоров'я чоловіка. – 2011. – №3. – С. 124–126.
8. Вакулєнко Г. А., Коханевич Є. В., Базарна А. В. Шляхи оптимізації лікування патології шийки матки // Здоров'я жінки. – 2003. – №1 (13). – С. 50–52.
9. Макагонова В. В., Корнієць Н. Г., Удовіка Н. О. Особливості перебігу передракових захворювань шийки матки у жінок та ефективності Протефлазиду у комплексі лікування // Збірник наукових праць «Проблеми екологічної та медичної генетики і клінічної імунології». – Київ – Луганськ – Харків, 2003. – С. 241–251.
10. Грищенко О. В., Бобрицька В. В., Пак С. А. Комплексна імуномодулююча терапія папіломавірусної інфекції у вагітних // Збірник наукових праць Асоціації акушерів гінекологів України. – К.: Інтермед, 2005. – С. 112–115.
11. Кишакевич І. Т. Сучасні аспекти лікування доброякісних процесів шийки матки, асоційованих з папіломавірусною інфекцією // Педіатрія, акушерство та гінекологія. – 2004. – № 2. – С. 116–118.

12. Маланчук Л. М., Зайкова Т. В., Флехнер В. М., Багній Н. І. Застосування препарату Протефлазид® у комплексному лікуванні дисплазій шийки матки на тлі папіломавірусної інфекції // Науково-практичний журнал «Вісник наукових досліджень». – 2003. – №4 (33). – С. 77–78.
13. Волошина Н. М., Петрова О. Ю., Кузнецова Т. П., Єлізарова Л. І., Кошлякова Є. С. Моніторинг шийки матки у вагітних // Здоров'я жінки. – 2007. – №2 (30). – С. 63–67.
14. Годлевська Н. А., Старовір А. В. Ефективність системного та місцевого застосування препарату Протефлазид® у лікуванні патології шийки матки, спричиненої папіломавірусною інфекцією // Здоров'я жінки. – 2012. – №3 (69). – С. 80–83.
15. Оцінка клінічної ефективності Протефлазиду в терапії хронічних запальних захворювань геніталій, ускладнених папіломавірусною інфекцією [Звіт] / ДУ «Інститут урології НАМН України» відділ сексопатології і андрології. – Київ, 2010. – С. 29.
16. Ліанці Е. І., Сафроннікова Н. Р., Редько Л. А., Мерабішвілі В. М. Ефективність натуральних імуномодуляторів у лікуванні захворювань, викликаних онкогенними вірусами: матеріали Всеросійської науково-практичної конференції з міжнародною участю // (2–3 вересня 2010 року). – СПб. – 2010. – С. 57–60.
17. Герасимова Т. В., Гопчук О. М. Застосування Протефлазиду в комплексній терапії порушень менструального циклу, спричинених вірусною інфекцією // Репродуктивне здоров'я жінки. – 2007. – №1 (30). – С. 76–79.
18. Галнікіна С. О. Клініко-патогенетичне обґрунтування лікування патології шкіри та слизових оболонок у жінок з постоваріоєктомічним синдромом: Автореф. дис. ... д-ра мед. наук: спец. 14.10.10 «Акушерство та гінекологія» / Галнікіна О. С.. – К., 2004. – С. 39.
19. Кишакевич І. Т. Активність лізоциму як критерій ефективності лікування жінок з фоновими захворюваннями шийки матки, асоційованими з вірусними інфекціями // Вісник наукових досліджень. – 2003. – №4 (33). – С. 58–60.
20. Волошина Н. М. Особливості перебігу папіломавірусної інфекції у вагітних (Клінічна лекція) // Репродуктивне здоров'я жінок. – 2006. – №3 (28). – С. 82–85.
21. Абдіраїмова Р. Б., Мамбетова М. Є., Абдурасимова З. А., Сатанова Х. М., Тлеубергенова У. Є., Ісабекова А. Ж. Протефлазид® у лікуванні папіломавірусних уrogenітальних інфекцій цій // Вісник ЮКДФА «Хабаршіси». – 2013. – №1 (62). – С. 129–131.
22. Бодян В. В. Нові методи медикаментозного лікування кондилом шийки матки, піхви та зовнішніх стативих органів / В. В. Бодян // Всеукраїнський медичний журнал «Хіст» [під ред. чл. кор. АПН України, д-ра мед. наук, професор Пішака В. П.]. – 2005. – №7. – С. 15.
23. Протефлазид® у терапії урогенітальної патології [рекомендації для лікарів]. – Київ, 2004. – С. 15.
24. Кишакевич І. Т., Кучеренко К. В. Методика застосування препарату Протефлазид® у терапії фонових захворювань шийки матки при папіломавірусній та герпетичних інфекціях // Інформаційний лист. – К., 2003. – №129. – С. 3.
25. Мамон А. А. Комплексна терапія хворих папіломатозновірусною інфекцією шкіри // Інформаційний лист. – Київ, 2004. – №125. – С. 3.
26. Курбанов Д. Д., Мусабєв Е. І., Аріпджанова Д. С. Протефлазид® – нове у лікуванні вірусних інфекцій (ВПЛ, ВПГ, ЦМГ) в акушерстві, гінекології та перинатології [Методичні рекомендації] / – Ташкент, 2004. – С. 14.
27. Волошина Н. М. Цервікальні інтраепітеліальні неоплазії (діагностика, лікування, профілактика) [Методичні рекомендації] / Волошина Н. М. – Запоріжжя, 2007. – С. 36.

28. Запорожан В. М., Марічереда В. Г., Дімчева Л. І. Застосування флавонових глікозидів у терапії генітальної папіломавірусної інфекції у жінок. / Journal of Education, Health and Sport formerly Journal of Health Sciences. – 2014. – №4 (12). – С. 93–104.
29. Порівняльна оцінка ефективності та переносимості препарату Протефлазід®, супозиторії виробництва ТОВ «ФармексГруп» та препарату Протефлазід®, краплі, виробництва ПАТ «Фітофарм» у пацієнток з дисплазією епітелію шийки матки, зумовленої папіломавірусною інфекцією [Звіт] / Національна медична академія з післядипломної освіти імені П. Л. Шупика. – К., 2015. – С. 78.
30. Волошина Н. М. Діагностика, лікування та профілактика передракових захворювань шийки матки [Методичні рекомендації] – Запоріжжя, 2003. – С. 24.
31. Волошина Н. М. Передракові захворювання шийки матки [Методичні рекомендації] – Запоріжжя. – 2005. – С. 8.
32. Методика застосування Протефлазиду у лікуванні дисплазій шийки матки // Інформаційний лист МОЗ України. – К., 2004. – С. 4.

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3.2. EVALUATION OF EFFICACY OF PROTEFLAZID® IN THE TREATMENT OF HPV-INFECTION: META- ANALYSIS OF LONG-TERM CLINICAL TRIALS RESULTS

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Abstract

To evaluate the clinical efficacy of PROTEFLAZID® in treatment of patients with urogenital diseases and cervical pathology caused by HPV infection a meta-analysis of clinical case-control trials, which involved more than 1,000 patients, was performed. The results showed that PROTEFLAZID® eliminates human papillomavirus from the body, prevents the recurrence of the disease, reduces the risk of virus-induced neoplastic processes.

Key words: meta-analysis, human papillomavirus, papillomaviral infection, cervical pathology, urogenital diseases, PROTEFLAZID®

Introduction

Human papillomavirus (HPV) is a widespread and highly variable group of viruses. Over the past decade, the diagnosis and treatment of HPV-associated diseases have remained pressing issues due to a sharp increase in incidence rates, high contagiousness, and the strong oncogenic potential of this pathogen across various age groups. HPV primarily targets epithelial cells of the skin and mucous membranes. The virus can exert either a productive or a transformative effect on the epithelium. A productive infection results in benign neoplasms – papillomas and condylomas of the skin and mucous membranes. In contrast, a transformative infection leads to cervical epithelial dysplasia, which may progress to cervical cancer [15]. While the overall incidence of clinically advanced or neglected cervical cancer is declining, a consistent upward trend is observed in the proportion of intraepithelial and microinvasive cervical cancers. This trend is most prominent among young women, with a notable characteristic of this oncopathology being its short latency period, which, according to some authors, may be less than 12 months.

A marked global increase in HPV infection rates has been observed in recent years. Over the past decade, the number of individuals diagnosed with human papillomavirus (HPV) infection has increased more than tenfold. Given the virus's predominant sexual transmission route, the highest prevalence is found among young, sexually active women. According to various studies, the HPV infection rate among individuals aged 16–29 ranges from 45% to 81% [16]. The spread of this sexually transmitted infection has reached epidemic levels. Cytological evidence of HPV infection is present in 88% of patients with cervical epithelial dysplasia, with infected cells most frequently (80.2%) localised in the transformation zone – the key site for epithelial cell differentiation in the cervix [19].

Genital HPV infection is among the most common infections globally, with a prevalence rate of 11.7% (95% confidence interval [CI]: 11.6–11.7). More than 70 HPV strains are associated with human disease, and it is estimated that approximately 630 million people worldwide are infected. The five most frequently detected high-risk (oncogenic) HPV genotypes are HPV-16 (3.2%), HPV-18 (1.4%), HPV-52 (0.9%), HPV-31 (0.8%), and HPV-58 (0.7%) [19].

However, these data only reflect the incidence of clinical manifestations of HPV and may not represent the full size of population-level infection, as subclinical and latent forms of HPV are not recorded.

HPV infections typically progress through several stages:

- 1) initial infection, characterised by the localisation of the virus to a defined anatomical site;
- 2) viral persistence, during which the viral genome remains in episomal form and viral particle production occurs alongside epithelial cell differentiation (secondary infection may also occur at this stage);
- 3) oncogenic transformations resulting from the interaction of viral oncogenes with cellular regulatory proteins following the integration of viral DNA into the host genome [17].

The diagnosis of typical manifestations of HPV infection is generally uncomplicated; however, early-stage detection presents significant challenges. The primary diagnostic modalities for HPV infection include cytological and histopathological examinations (with identification of koilocytes in biopsy specimens), complemented by polymerase chain reaction (PCR)-based assays for accurate viral genotyping.

For over three decades, extensive discourse in the scientific literature has focused on identifying the optimum therapeutic approach for this patient population. The treatment options remain controversial and unstandardised, primarily due to the lack of antiviral agents with direct activity against HPV. Promising outcomes of early-stage tumours treatment, characterised by a progressive reduction in the number of surgical interventions, have driven the emergence of a novel subspecialty in gynaecologic oncology – organ-preserving treatment [20, 22].

In recent years, there has been a notable trend toward unjustified and in some cases, overly aggressive treatment of women with HPV, which often results in long-term complications and disease recurrence.

The standard approach to treating cervical epithelial dysplasia associated with HPV infection involves surgical procedures, such as electrocoagulation, cryodestruction, and similar techniques. Therapeutic strategies often incorporate agents that induce chemically mediated

necrosis of exophytic HPV lesions. To prevent recurrence, recombinant interferon formulations and endogenous interferon inducers are commonly used.

Until recently, no specific treatment for HPV infection was available in global clinical practice. However, after extensive research, Ukrainian scientists succeeded in developing a pharmaceutical agent, PROTEFLAZID®, with direct antiviral activity against HPV.

PROTEFLAZID® demonstrates specific antiviral activity against HPV. In experimental *in vitro* models of oncogenic HPV, the active ingredient has been shown to inhibit HPV replication by 2 log ID₅₀. According to cytological analysis data, PROTEFLAZID® suppresses both the proliferative and destructive effects of HPV on host cells. The mechanism of the direct antiviral activity of PROTEFLAZID® involves inhibition of viral DNA synthesis in infected cells, mediated through the suppression of virus-specific enzymes – DNA polymerase and thymidine kinase.

PROTEFLAZID® promotes the synthesis of endogenous α - and γ -interferons to physiologically active levels (without refractoriness), thereby enhancing nonspecific resistance to viral and bacterial infections and contributing to the normalisation of immune status. This product also prevents the accumulation of lipid peroxidation products (suppresses free radical reactions) and acts as an apoptosis modulator, facilitating the elimination of infected cells.

Clinical trials have demonstrated that topical administration of PROTEFLAZID® (suppositories and vaginal tampons soaked in PROTEFLAZID® solution) contributes to the restoration of the barrier function of the vaginal and cervical mucosa by normalising key components of local immunity (secretory immunoglobulin A, lysozyme, and complement component C₃) [21].

Study Objectives: conducting a meta-analysis and assessing the clinical efficacy of PROTEFLAZID® in the treatment of patients with urogenital diseases and cervical pathology associated with HPV infection.

Materials and Methods

Information on clinical trials investigating the efficacy of the medicinal product PROTEFLAZID® in women with urogenital diseases and cervical pathology associated with HPV infection was obtained through structured searches of online databases. A list of relevant clinical publications from 2002 to 2015 is presented in Table 1. The key stages of the meta-analysis were conducted following established standards in the field, integrating data from multiple comparable studies into a single outcome, thereby enabling the assessment of treatment efficacy across a substantially larger sample size than that of any individual trial.

Data Analysis Tools

To ensure a higher level of reliability of the results, two specialised statistical software packages were used in parallel: RevMan and Comprehensive Meta-Analysis.

Inclusion Criteria

Trials included in the meta-analysis met the following criteria:

1. A case-control trial focused on assessing the clinical efficacy of PROTEFLAZID® in the treatment of patients with urogenital diseases and cervical pathology associated with HPV infection.
2. Confirmed clinical diagnosis of HPV-related cervical pathology or urogenital disease in all enrolled participants.
3. Completeness of published data on PROTEFLAZID® efficacy.

Table 1: List of publications reflecting the efficacy of PROTEFLAZID® in the treatment of patients with urogenital diseases and cervical pathology caused by HPV

Author	Year	Total number of patients	Scope of PROTEFLAZID® application	Outcomes of clinical trials
Radionov V. H. [17]	2002	39	Clinical manifestations of HPV (condylomas)	Significant reduction in the recurrence of condylomatous lesions
Vakulenko H. A. [20]	2003	599	Cervical pathology	Significant reduction in HPV replication, improvements on colposcopy
Makahonova V. V. [13]	2003	48	Precancerous cervical diseases	Significant reduction in HPV replication, improvements on colposcopy
Malanchuk L. M. [4]	2003	22	Cervical dysplasia with HPV	Significant regression of cervical dysplasia
Palichuk O. V. [9]	2004	11	The initial stage of cervical cancer	Significant reduction in HPV replication, improvements on colposcopy
Kyshakevych I. T. [8]	2004	120	Benign cervical lesions	Significant reduction in HPV replication, improvements on colposcopy
Kyshakevych I. T. [1]	2003	44	Cervical pathology	Complete restoration of serum lysozyme activity and cervical mucus
Galnikina S. O. [2]	2004	356	Skin and mucous membranes pathology in women with post-ovariectomy syndrome	Eliminating immune system failure
Hryshchenko O. V. [10]	2005	33	HPV in pregnant women	Significant reduction in HPV replication, no recurrences
Lisovyi V. M. [21]	2006	34	Urogenital HPV	Significant reduction in HPV replication, elimination of leukaemia and neutropenia in the peripheral blood
Voloshyna N. [12]	2007	136	Cervical pathology in pregnant women	Significant reduction in HPV replication
Herasymova T. V. [5]	2007	150	Menstrual disorders caused by a viral infection	Significant reduction in HPV replication, improvements on colposcopy
Laliantsi E. I. [23]	2010	625	Virus-associated urogenital pathologies	Significant reduction in HPV replication, regression of cervical dysplasia
Romashenko O. V. [14]	2010	32	Chronic inflammatory genital diseases, complicated by HPV	Significant reduction in HPV replication, improvements on colposcopy
Romaniuk M. H. [7]	2011	187	HPV in men	Significant reduction in the recurrence of condylomatous lesions, significant reduction in HPV replication
Hodlevska N. A. [3]	2012	32	Cervical pathology	Significant reduction in HPV replication, improvements on colposcopy
Abdyraimova R. B. [18]	2013	12	Papillomavirus urogenital infection	Significant reduction in HPV replication, elimination of leukaemia and neutropenia in the peripheral blood
Zaporozhan V. M. [6]	2014	80	Genital papillomavirus infection	Significant reduction in HPV replication
Kaminskiy V. V. [21]	2015	76	Cervical epithelial dysplasia caused by HPV	Significant reduction in HPV replication

Based on the aforementioned inclusion criteria, eight case-control clinical trials were included in the meta-analysis, and eleven were excluded. The selected publications covered the period from 2002 to 2015.

Among the eight included trials, the basic treatment group comprised a total of 539 female patients with urogenital diseases and cervical pathology associated with HPV infection. These patients received PROTEFLAZID® either orally, according to a standardized dosing regimen, or via local applications and vaginal tampons soaked in the drug solution. The control group included 498 participants. All patients in both groups underwent comprehensive pre- and post-treatment assessment, which included standard clinical and laboratory tests, pelvic ultrasound, assessments of cellular and humoral immunity, urethroscopy, and colposcopy. The principal diagnostic method was PCR testing with genotyping of HPV types 16, 18, 31, and 33.

The meta-analysis was conducted based on three key outcome measures:

- 1. The recurrence rate of condylomatous lesions.
- 2. The detection rate of HPV by PCR.
- 3. The rate of HPV clearance (or significant reduction in viral replication), as determined by PCR.

A total of 106 female patients with urogenital diseases and cervical pathology associated with HPV infection were analysed for the outcome measure of condylomatous lesion recurrence, with 65 patients in the treatment group and 41 in the control group.

A total of 965 female patients with urogenital diseases and cervical pathology associated with HPV infection were analysed for the outcome measure of HPV detection rate by PCR, with 494 patients in the treatment group and 471 in the control group (see Table 2).

Table 2: HPV detection rate by PCR

Author, year	Treatment group		Control group	
	Number of patients with HPV detected by PCR	Total number of patients	Number of patients with HPV detected by PCR	Total number of patients
Makahonova V. V., 2003	1	9	5	12
Malanchuk L. M., 2003	1	12	4	10
Lisovyi V. M., 2006	2	20	8	14
Laliantsi E. I., 2010	34	307	96	318
Romaniuk M. H., 2011	27	108	64	79
Kaminskiy V. V., 2015	0	38	3	38
Total	65	494	180	471

A total of 1,037 female patients with urogenital diseases and cervical pathology associated with HPV infection were analysed for the outcome measure of HPV clearance (or significant reduction in viral replication) rate, with 539 patients in the treatment group and 498 in the control group.

Results and Discussion

Data pooling for analysis. The statistical data of the meta-analysis based on the calculated odds ratio (OR) for the recurrence rate of condylomatous lesions is shown in Table 3.

Figure 1 presents a Forest plot illustrating the pooled ORs from the meta-analysis of condylomatous lesions recurrence in the treatment and control groups.

The χ^2 test ($P=0.34$) and the I^2 statistic ($I^2=6\%$) indicate homogeneity of study data, supporting the use of a fixed-effect model. The P-value yielded in the F-test ($P=0.003$) demonstrates the statistical significance of the observed effect.

An OR of 0.18 suggests that patients in the treatment group had a 5.5-fold lower likelihood of condylomatous lesion recurrence compared to those in the control group.

The study by Hryshchenko O. V. et al. (2005) contributed the greatest weight to the analysis, reporting that treatment with PROTEFLAZID® led to regression of condylomatous lesions, as evidenced by a reduction in the size of skin and mucous membrane lesions [10]. An analysis of leukograms conducted by Radionov V. H. et al. (2002) demonstrated a positive effect of PROTEFLAZID® on the innate immune resistance in patients from the treatment group [17]. According to Lisovyi V. M. et al. (2006), the drug increases treatment efficacy by 15–17%, and its beneficial effect is sustained over time. Additionally, it was associated with a reduced risk of transmission of the infection to sexual partners, as well as a 47–50% decrease in the risk of virus-induced neoplastic transformations [21].

The statistical data of the meta-analysis based on the calculated ORs for the HPV detection rate by PCR is shown in Table 4.

Figure 2 presents a Forest plot illustrating the pooled ORs from the meta-analysis of HPV detection rate by PCR in the treatment and control groups.

The χ^2 test ($P=0.06$) indicates a homogeneity of the study data, and the I^2 statistic ($I^2=53\%$) suggests moderate heterogeneity. Therefore, to ensure reliability, a random-effect model was applied. The P-value yielded in the F-test ($P=0.00001$) demonstrates the statistical significance of the observed effect.

An OR of 0.15 suggests that patients in the treatment group had a 6.7-fold lower likelihood of HPV detection by PCR compared to those in the control group.

The greatest weight to this analysis had the studies by Laliantsi E. I. and Romaniuk M. H. In particular, Laliantsi E. I. et al. (2010) demonstrated high clinical efficacy of PROTEFLAZID® in the treatment of virus-associated vulvovaginal pathology [23]. In a clinical trial by Romaniuk M. H. et al. (2001), courses of PROTEFLAZID® treatment led to a decrease in the carriage rate of high-risk oncogenic HPV strains [7].

Table 3: The statistical data of the meta-analysis based on the calculated ORs for the recurrence rate of condylomatous lesions

Author, year	OR	Lower limit	Upper limit	Standard error
Radionov V. H., 2002	0.393	0.074	2.077	0.850
Hryshchenko O. V., 2005	0.032	0.002	0.645	1.537
Lisovyi V. M., 2006	0.193	0.018	2.089	1.215
Fixed effect	0.211	0.061	0.731	-

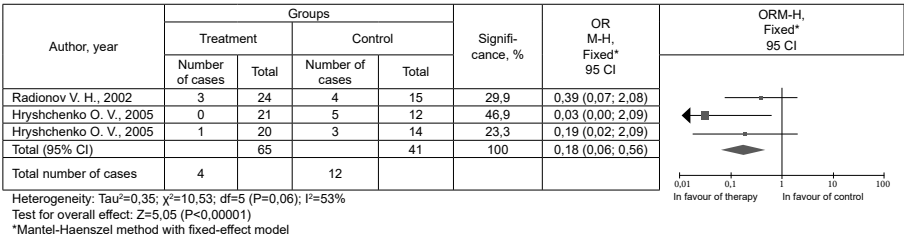


Figure 1: The pooled ORs from the meta-analysis of condylomatous lesions recurrence in the treatment and control groups

Table 4: The statistical data of the meta-analysis based on the calculated ORs for the HPV detection rate by PCR

Author, year	OR	Lower limit	Upper limit	Standard error
Makahonova V. V., 2003	0.175	0.016	1.881	1.212
Malanchuk L. M., 2003	0.136	0.012	1.513	1.228
Lisovyi V. M., 2006	0.083	0.014	0.506	0.920
Laliantsi E. I., 2010	0.288	0.187	0.442	0.219
Romaniuk M. H., 2011	0.078	0.038	0.159	0.363
Kaminskiy V. V., 2015	0.132	0.007	2.641	1.530
Random effect	0.145	0.069	0.307	-

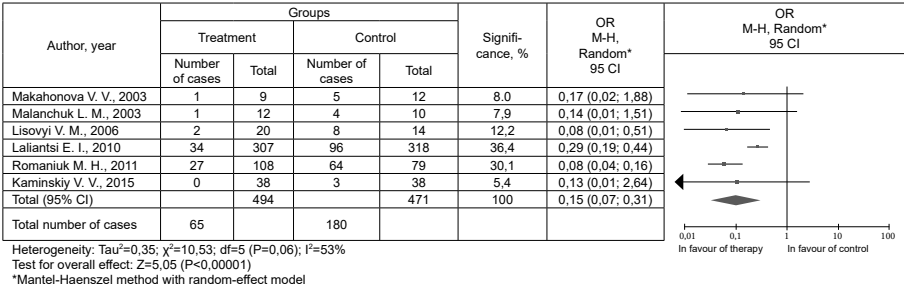


Figure 2: The pooled ORs from the meta-analysis of HPV detection rate by PCR in the treatment and control groups

Kaminskiy V. V. (2015) reported that treatment with PROTEFLAZID® led to the resolution of vaginal microbiota dysbiosis and an improvement in local immunity [21].

The statistical data of the meta-analysis based on the calculated ORs for the HPV clearance (significant reduction in viral replication), as determined by PCR.

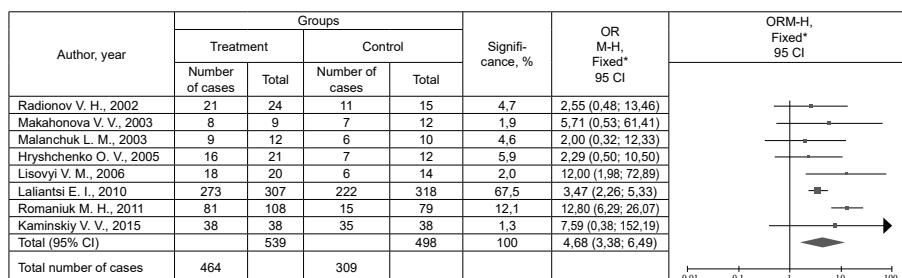
Figure 3 presents a Forest plot illustrating the pooled ORs from the meta-analysis of HPV clearance (significant reduction in viral replication), as determined by PCR, in the treatment and control groups.

The χ^2 test ($P=0.07$) and the I^2 statistic ($I^2=46\%$) indicate homogeneity of study data, supporting the use of a fixed-effect model. The P -value yielded in the F -test ($P=0.00001$) demonstrates the statistical significance of the observed effect.

An OR of 4.68 suggests that patients in the treatment group had a 4.7-fold higher likelihood of HPV clearance (significant reduction in viral replication), as determined by PCR, compared to those in the control group.

Table 5: The statistical data of the meta-analysis based on the calculated ORs for the HPV clearance (significant reduction in viral replication), as determined by PCR

Author, year	OR	Lower limit	Upper limit	Standard error
Radionov V. H., 2002	2.545	0.418	13.458	0.850
Makahonova V. V., 2003	5.714	0.532	61.410	1.212
Malanchuk L. M., 2003	2.000	0.324	12.329	0.928
Hryshchenko O. V., 2005	2.286	0.497	10.503	0.778
Lisovyi V. M., 2006	12.000	1.976	72.890	0.920
Laliantsi E. I., 2010	3.472	2.260	5.334	0.219
Romaniuk M. H., 2011	12.800	6.285	26.067	0.363
Kaminskiy V. V., 2015	7.592	0.379	152.187	1.530
Fixed effect	4.681	3.346	6.495	-



Heterogeneity: $\chi^2=12,92$; $df=7$ ($P=0,07$); $I^2=46\%$

Test for overall effect: $Z=9,28$ ($P<0,00001$)

*Mantel-Haenszel method with fixed-effect model

Figure 3: The pooled ORs from the meta-analysis of HPV clearance (significant reduction in viral replication), as determined by PCR, in the treatment and control groups

The studies by Laliantsi E. I. and Romaniuk M. H. are of particular relevance. Romaniuk M. H. et al. (2001) reported a decrease in the severity of dysplasia in recurrent condylomas. The authors consider oral courses of PROTEFLAZID® in combination with local application of the drug solution to be a promising therapeutic strategy, which may induce condyloma regression without the need for surgical intervention [7].

Makahonova V. V. et al. (2003) reported that, at Day 21 of treatment, HPV replication in multilayer epithelial cells was eliminated in 88.88% of patients with HPV infection in the

PROTEFLAZID® group and 58.34% of patients in the control group [13]. CO₂ laser vaporisation of pathological areas resulted in complete cervical epithelialization, confirmed by colposcopy.

In a clinical trial by Malanchuk L. M. et al. (2003), regression of dysplasia was observed in 60% of patients treated orally with PROTEFLAZID® and 83.3% of patients receiving combined oral treatment and vaginal tampons soaked with the drug solution [4]. **Thus, simultaneous oral and intravaginal administration of the drug significantly enhances therapeutic efficacy.**

Sensitivity Analysis

Sensitivity analysis was performed to evaluate the impact of each individual study on the pooled results by systematically excluding studies one at a time. The sensitivity analysis indicated that no single study significantly influenced the pooled data, suggesting statistically robust findings.

Assessment of Publication Bias

Funnel plots were used to evaluate publication bias in the studies taken for analysis (Figures 4–6). Nearly all standard error values in Figures 4–6 lie within the funnel region, indicating the absence of systematic bias.

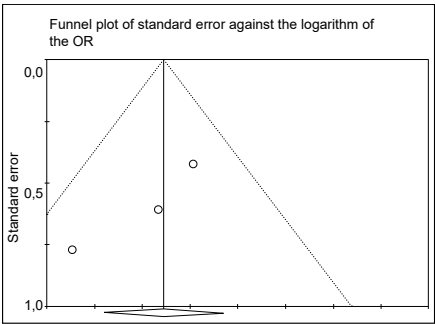


Figure 4: Funnel plot for the recurrence rate of condylomatous lesions

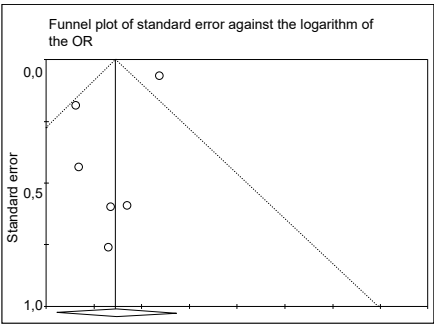


Figure 5: Funnel plot for the HPV detection rate by PCR

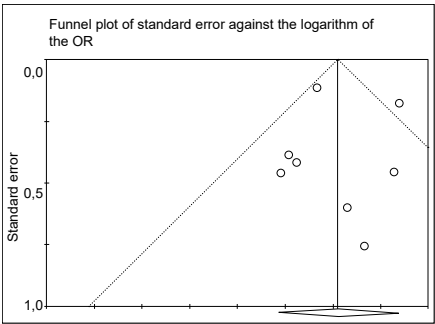


Figure 6: Funnel plot for the HPV clearance (significant reduction in viral replication), as determined by PCR

Conclusions

According to the meta-analysis, patients in the treatment groups had 5.5-fold lower likelihood of condylomatous lesion recurrence, 6.7-fold lower likelihood of HPV detection by PCR, and 4.7-fold higher likelihood of HPV clearance (significant reduction in viral replication), as determined by PCR, compared to controls.

These findings from the meta-analysis support the high clinical efficacy of PROTEFLAZID® in the treatment of patients with HPV-associated urogenital diseases and cervical pathology. PROTEFLAZID® facilitates the elimination of HPV from the host, thereby preventing disease recurrence and reducing the risk of virus-induced neoplastic transformations.

The medicinal product PROTEFLAZID®, in the form of oral drops and suppositories, may be recommended as an effective antiviral agent for the management of HPV-associated cervical pathology and urogenital diseases as part of etiopathogenetic therapy in the acute phase, during recovery, and in persistent HPV infection.

References:

1. Кишакевич І. Т. Активність лізоциму як критерій ефективності лікування жінок з фоновими захворюваннями шийки матки, асоційованими з вірусними інфекціями // Вісник наукових досліджень. – 2003. – №4 (33). – С. 58–60.
2. Галникіна С. О. Клініко-патогенетичне обґрунтування лікування патології шкіри та слизових оболонок у жінок з постоваріоєктомічним синдромом: Автореф. дис. д-ра мед.наук: спец. 14.10.10 «Акушерство та гінекологія» / – К., 2004. – С. 39.
3. Годлевська Н. А., Старовір А. В. Ефективність системного та місцевого застосування препарату протекфлазид у лікуванні патології шийки матки, спричиненої папіломавірусною інфекцією // Здоров'я жінки. – 2012. – №3 (69). – С. 80–83.
4. Маланчук Л. М., Зайкова Т. В., Флехнер В. М., Багній Н. І. Застосування препарату Протекфлазид® у комплексному лікуванні дисплазій шийки матки на фоні папіломавірусної інфекції // Науково-практичний журнал «Вісник наукових досліджень». – 2003. – №4 (33). – С. 77–78.
5. Герасимова Т. В., Гопчук О. М. Застосування Протекфлазиду в комплексній терапії порушень менструального циклу, спричинених вірусною інфекцією // Репродуктивне здоров'я жінки. – 2007. – №1 (30). – С. 76–79.
6. Запорожан В. М., Маричереда В. Г., Дімчева Л. І. Застосування флавонових глікозидів у терапії генітальної папіломавірусної інфекції у жінок / Journal of Education, Health and Sport formerly Journal of Health Sciences. – 2014. – №4 (12). – С. 93–104.
7. Романюк М. Г., Корнієнко А. М., Аксьонов П. В. Використання Протекфлазиду для лікування папіломавірусної інфекції для чоловіків // Здоров'я чоловіка. – 2011. – №3. – С. 124–126.
8. Кишакевич І. Т. Сучасні аспекти лікування доброякісних процесів шийки матки, асоційованих з папіломавірусною інфекцією // Педіатрія, акушерство та гінекологія. – 2004. – №2. – С. 116–118.
9. Палійчук О. В. Клінічний досвід використання препарату Протекфлазид® у комплексному лікуванні хворих з початковими формами раку шийки матки // Здоров'я жінки. – 2004. – № 2 (18). – С. 49–50.
10. Грищенко О. В., Бобрицька В. В., Пак С. А. Комплексна імунотулююча терапія папіломавірусної інфекції у вагітних // Збірник наукових праць Асоціації акушерів-гінекологів України. – К.: Інтермед, 2005. – С. 112–115.

11. Кишакевич І. Т., Кучеренко К. В. Методика застосування препарату протекфлазид в терапії фонових захворювань шийки матки при папіломавірусній та герпетичних інфекціях // Інформаційний лист. – К., 2003. – №129. – С. 3.
12. Волина Н. Н., Петрова О. Ю., Кузнецова Т. П., Єлізарова Л. І., Шлакова Є. С. Моніторинг шийки матки у вагітних // Здоров'я жінки. – 2007. – №2 (30). – С. 63–67.
13. Макагонова В. В., Корнієць Н. Г., Удовіка Н. О. Особливості перебігу передракових захворювань шийки матки у жінок та ефективності протекфлазиду в комплексі лікування // Збірник наукових праць «Проблеми екологічної та медичної генетики і клінічної імунології». – Київ – Луганськ – Харків. – 2003. – С. 241–251.
14. Оцінка клінічної ефективності протекфлазиду в терапії хронічних запальних захворювань геніталій, ускладнених папіломавірусною інфекцією [Звіт] / ДУ «Інститут урології НАМН України», відділ сексопатології і андрології. – Київ, 2010. – С. 29.
15. Фоляк Е. В., Соколова Т. М., Макаров К. Ю., Якимова А. В., Мухамедшина В. Р., Усова А. В. Папіломавірусна інфекція уrogenітального тракту жінок (епідеміологія, клініко-патогенетичні особливості, методи діагностики, лікування, профілактика): інформаційно-методичний посібник. – Вектор-Бест. – 2010. – С. 88.
16. Волошина Н. М. Передракові захворювання шийки матки [Методичні рекомендації]. – Запоріжжя. – 2005. – С. 8.
17. Радіонов В. Г., Шведюк С. В., Семіряд Ю. В., Радіонов Д. В., Шеданія І. Є., Коротін Ю. Л. Застосування Протекфлазиду в комплексній терапії папіломавірусної інфекції // Український журнал дерматології, венерології, косметології. – 2002. – No 4. – С. 86–90.
18. Абдіраїмова Р. Б., Мамбетова М. Е., Абдурасімова З. А., Саганова Х. М., Тлеубергенова У. Е., Ісабекова А. Ж. Протекфлазид® в лікуванні папіломавірусних уrogenітальних інфекцій // Вісник ЮКГФА «Хабаршиси». – 2013. – No 1 (62). – С. 129–131.
19. Камінський В. В., Шалько М. Н., Воробйова Л. І., Ромащенко О. В., Гриневич О. Й. Протекфлазид®: специфічна активність у доклінічних дослідженнях та безпека застосування в клінічній практиці при захворюваннях, спричинених вірусом папіломи людини (систематичний огляд) // Здоров'я жінки. – No 2 (99). – 2015. – С. 128–138.
20. Вакулєнко Г. А., Коханевич Е. В., Базарна А. В. Шляхи оптимізації лікування патології шийки матки // Здоров'я жінки. – 2003. – No 1 (13). – С. 50–52.
21. Порівняльна оцінка ефективності та переносимості препарату Протекфлазид®, супозиторії виробництва ТОВ «Фармекс Груп» та «Протекфлазид®», краплі, виробництва ПАТ «Фітофарм» у хворих з дисплазією епітелію шийки матки внаслідок папіломавірусної інфекції [Звіт] / Національна медична академія післядипломної освіти імені П. Л. Шупика. – Київ, 2015. – С. 78.
22. Флетчер Р., Флетчер С., Вагнер Е. Клінічна епідеміологія: основи доказової медицини. Видавництво «Медіа Сфера», 1998. – С. 331.
23. Лаліанці Е. І., Сафроннікова Н. Р., Редько Л. А., Мерабішвілі В. М. Ефективність природних імуномодуляторів у лікуванні захворювань, спричинених онкогенними вірусами: матеріали науково-практичної конференції з міжнародною участю // (2-3 вересня 2010 р.). – 2010. – С. 57–60.
24. Лісовий В. Н., Яковлева Е. В. Ефективність препарату Протекфлазид в комплексній терапії хворих на уrogenітальну папіломавірусну інфекцію // Здоров'я чоловіка. – 2006. – No 2. – С. 230–232.
25. Bero L., Rennie D. The Cochrane Collaboration: preparing, maintaining and disseminating systematic reviews of the effects of health care. JAMA 1995; 274:1935–8.

Reference: V. V. Kaminskiy, M. N. Shalko, V. S. Michailov, M. V. Lesnikova, L. A. Yashchenko, O. Y. Hrynevych, T. A. Broun (2015). Evaluation of Efficacy of PROTEFLAZID® in the Treatment of HPV-Infection: Meta-Analysis of Long-Term Clinical Trials Results. Medical Aspects of Women's Health, 5 (92), 5–14.

3.3. COMPARATIVE EFFECTIVENESS OF DIFFERENT PROTEFLAZID® FORMULATIONS IN THE TREATMENT OF MILD TO MODERATE CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN) ASSOCIATED WITH GENITAL HPV INFECTION

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Abstract

The results of the evaluation of clinical efficacy include the use of combination therapy with a herbal preparation of direct antiviral and immunomodulatory in the form of suppositories and drops for oral use in the treatment of mild and moderate degrees of cervical intraepithelial neoplasia, which in turn are due to genital papillomaviral infection. The objective: to compare the effectiveness of treatment with CIN I – II, caused by the human papillomavirus (HPV), when PROTEFLAZID® is used in the form of monotherapy systemically, locally and simultaneously systemically and locally.

Materials and methods. The study involved 90 women of reproductive age with cervical intraepithelial neoplasia of the cervix of varying degrees (CIN I – II), due to PVI. All patients underwent examination and treatment at the Kyiv City Center for Reproductive and Perinatal Medicine (CMRCC) and were divided into three groups. Group I included 30 (33.33%) patients treated with PROTEFLAZID® in the form of drops according to the following scheme: 15 drops 2 times a day for 3 months – without interruption. Group II included 30 (33.33%) women who received topical treatment with the use of PROTEFLAZID® suppositories according to the following scheme: 1 suppository 2 times a day for 14 days of each month during the intermenstrual period; course – 3 months Treatment was started immediately after menstruation. At the time of menstruation did a break in treatment. Group III consisted of 30 (33.3%) surveyed women who received both suppositories of PROTEFLAZID® – 1 suppository twice a day for 14 days; course – 3 months (monthly for 14 days per intermenstrual period), and at the same time prescribed a drop form of the drug PROTEFLAZID® according to the scheme: 15 drops 2 times a day for 3 months – without a break. Considering that the process of passing one infected cell from the basal layer to the superficial can last up to 3 months, we chose a long-term treatment – a three-month course in order to eliminate the virus and prevent the recurrence of HPV.

Results. Systemic use of the drug PROTEFLAZID® drops (per os) in the treatment of mild to moderate neoplasia leads to improvement of the cervical epithelium in 60% of cases and eradication of HPV in 73.3%

of patients. Local application of the drug PROTEFLAZID® suppositories with CIN I – II normalizes the cytological picture or leads to the transition of CIN II to CIN I in 63.3% of women; HPV DNA after treatment was not detected in 76.7% of cases. Simultaneous local and systemic use of PROTEFLAZID® suppositories and PROTEFLAZID® drops for 3 months in the treatment of CIN I, CIN II, associated with PVI, led to regression of CIN I to normal and CIN I in CIN I in 83.3% of patients; HPV DNA after treatment was not detected in 90.0% of cases.

Conclusion. PROTEFLAZID® can be recommended for use in practice as a safe and effective drug for treating patients with CIN I and CIN II due to HPV in the form of monotherapy, especially in the simultaneous systemic and local application of PROTEFLAZID® (suppositories+drops), which will provide a high therapeutic efficiency.

Key words: dysplasia, cervical intraepithelial neoplasia, papillomavirus infection, immunomodulatory therapy, antiviral therapy.

Introduction

Cervical cancer (CC) is the seventh most common cancer among women in Europe, accounting for approximately 3.8% of all cancer cases [14]. Globally, it is the second most common cancer affecting women [15]. The majority of cervical cancer cases are diagnosed in less developed regions – low- and middle-income countries – and in high-income countries with screening programs in place, this disease is observed less frequently [1, 14, 15, 16].

According to the National Cancer Registry, cervical cancer ranks fifth in the structure of oncological diseases among women in Ukraine. Currently, the incidence of cervical cancer in Ukraine is 20 cases per 100,000 women [17].

Cervical cancer is preceded by a long phase of precancerous changes called cervical intraepithelial neoplasia (CIN).

CIN is typically localised in the area of cervical epithelial transformation and is associated with persistent infection caused by human papillomavirus (HPV).

In recent years, most oncologists have been using the classification of neoplasia proposed by R.M. Richart (1968). According to this classification, neoplasia is morphologically categorised into three grades, depending on the severity of abnormal changes: mild (CIN 1), moderate (CIN 2) and severe (CIN 3).

CIN 1 is characterised by polymorphism of cellular elements with pronounced nuclear hyperchromia and a high nuclear/cytoplasmic (N/C) ratio, all of which are observed in the superficial one-third of the stratified squamous epithelium [4, 5].

In CIN 2, the process progresses, with cellular atypia and numerous mitoses present in the half of the epithelial layer from the basement membrane.

CIN 3 is characterised by cellular atypia involving two-thirds of the epithelial thickness, in contrast to carcinoma in situ, where atypia extends throughout the full thickness of the epithelium, from the basal to the superficial layer.

The differential diagnosis between severe dysplasia and invasive cancer in cytological specimens can be challenging. To address this, the Bethesda System introduced the category of ‘high-grade squamous intraepithelial lesions’ (HSIL), which includes moderate, severe dysplasia (CIN 2, CIN 3), and cancer in situ.

Every year, more than 30 million new cases of mild dysplasia of the cervical squamous epithelium and over 10 million new cases of moderate to severe dysplasia are diagnosed worldwide [8]. Unfortunately, in Ukraine, the National Cancer Registry does not track the incidence

of precancerous cervical lesions, so the available data are inconsistent, as incidence estimates are based on individual studies and range from 18% to 43% of all cervical pathology.

Cervical intraepithelial lesions represent a stage in the process of cervical carcinogenesis.

There are two prevailing concepts regarding the development of invasive cervical cancer in the presence of HPV infection:

The first (classical) concept reflects the stepwise progression of CIN 1, 2, and 3 into invasive cancer (the majority of cases).

The second concept suggests that high-grade squamous intraepithelial lesions (CIN 2-3) can develop without a preceding CIN 1 stage [2-4].

Timely diagnosis and treatment of CIN is the prevention of invasive cervical cancer.

The diagnosis of cervical epithelial dysplasia does not imply inevitable progression to cervical cancer. In fact, more than 74% of moderate dysplasia cases regress spontaneously within 5 years (Holowaty P. et al., 1999). Analysis of the outcomes of dysplastic changes in the cervical squamous epithelium showed that in CIN 1, regression occurs in 57% of patients, persistence in 32%, progression in 11%, and invasive cervical cancer develops in 1% of cases. By contrast, in CIN 3, regression is observed in 32% of patients, and malignant transformation occurs in more than 12% of patients [10].

According to other studies, 91% of mild cervical dysplasia cases detected by Pap smear regress spontaneously within 36 months, and 3% progress to severe dysplasia [11].

To date, a large number of studies have shown that cervical epithelial dysplasia and cervical cancer develop in the presence of human papillomavirus (HPV) infection.

HPV infection is the primary and most significant risk factor for the development of cervical cancer. In fact, HPV genotypes are detected in 99.7% of cervical cancer cases [12]. The virus targets epithelial layers of the skin and mucous membranes. The transforming activity of HPV leads to the development of cervical intraepithelial neoplasia (CIN), which may progress to cervical cancer [6].

Certain high-risk (HR) types of HPV have a greater potential to induce cervical epithelial transformation and promote malignant progression.

HPV type 16 is the most oncogenic genotype worldwide, detected in almost 50% of cervical cancer cases. Type 18 is the second most common, found in 15% of patients with cervical cancer. HPV types 31 and 45 rank third in prevalence, collectively accounting for 10% of cervical cancer cases [12, 21, 22].

In Ukraine, HPV-16 is also the most common genotype across all regions and patient age groups. An epidemiological feature of HPV infection in Ukraine is the high prevalence of HPV genotypes 53, 31, 33, and 68, whereas genotype 18 ranks only ninth. The risk of HPV persistence and progression of cervical epithelial dysplasia increases with the simultaneous detection of multiple HPV genotypes with a high viral load. Both high- and low-risk HPV genotypes can act as etiological factors in the development of CIN 1 and CIN 2 [2, 23].

Current national and international literature extensively reports that HPV infection occurs at the level of immature epithelial cells of the skin and mucous membranes (basal layer). As these cells migrate towards the epithelial surface, they undergo differentiation. Active HPV replication occurs specifically in differentiated cells. The transit time of an infected cell from the basal to the superficial epithelial layer may last up to 3 months (Stanley, Margaret A., 2012). This fact necessitates prolonged HPV therapy for at least 3 months.

The subsequent progression of HPV infection is strongly influenced by a decline in immune defence and dysregulation of apoptosis.

The immune system's failure to control the virus leads to persistent HPV infection in the cervical epithelium. The viral DNA integrates into the DNA of epithelial cells, initiating synthesis of oncoproteins E6 and E7. HPV oncoproteins E6 and E7 bind to cellular tumour suppressor proteins p53 and pRB. This interaction blocks apoptosis (programmed death) of infected cells and disrupts proliferation control. As a result, the altered cells divide uncontrollably and rapidly, forming a clone of tumour cells.

It should be noted that HPV infection of epithelial cells is a necessary but not sufficient condition for the development of cancer. Additional factors contributing to the formation of irreversible neoplasia include active E6 and E7 genes of high-risk HPV types; induction of metabolic pathways converting estradiol to 16-OH; and induction of multiple chromosomal DNA damages in the infected cells, ultimately leading to malignant transformation [18].

Unfortunately, despite advances in understanding, diagnosis, and treatment of precancerous cervical conditions, more than 500,000 women worldwide are diagnosed with invasive cervical cancer annually, and 274,000 die from this disease [9]. In Ukraine, the total number of women living with a diagnosis of cervical cancer as of 2016 was 52,157, and 1,687 women died from cervical cancer in 2015 [17].

Reported incidence and mortality rates emphasise the need to improve the management and treatment strategies for CIN, particularly by avoiding overdiagnosis and unnecessary invasive interventions in young women, which may compromise their reproductive potential.

Although ablative treatment methods have demonstrated clinical efficacy, they are invasive and may negatively affect future fertility. Conversely, prolonged active surveillance or a watchful waiting approach carries the risk of disease progression. Therefore, identifying a conservative and safe therapeutic strategy for CIN 1–2 remains a relevant clinical goal.

A Ukrainian antiviral agent, PROTEFLAZID® (produced by SMC “Ecopharm” LTD), offers a promising approach to the management of HPV-associated CIN 1 and CIN 2 as it exhibits a direct antiviral effect against viral DNA, including HPV. The active substances, flavonoids extracted from *Herba Deschampsia caespitosa* L. and *Herba Calamagrostis epigeios* L., inhibit polymerases (HPV-specific enzymes) within infected cells. Suppression of these enzymes halts viral DNA replication, thereby preventing further proliferation.

PROTEFLAZID® has a specific direct antiviral activity against HPV. This has been demonstrated in *in vitro* studies evaluating its specific antiviral activity against HPV, conducted at the L. V. Hromashevskyi Institute of Epidemiology and Infectious Diseases in 2010 under the supervision of Dr. S. L. Rybalko. These *in vitro* experiments showed a reduction in the infectious titer by more than 2 lg [20].

Therefore, PROTEFLAZID® provides etiotropic therapy by acting directly against HPV.

PROTEFLAZID® also has immunotropic properties – it induces the synthesis of endogenous α - and γ -interferons to physiologically active levels without triggering refractoriness – a condition in which immune cells fail to produce interferons in response to repeated stimulation by an inducer. Refractoriness compromises the first line of antiviral defence, thereby increasing the risk of infectious complications. Therefore, PROTEFLAZID® can be administered over prolonged periods (which is particularly important in the treatment of persistent HPV infection) without the risk of immune exhaustion.

In addition to inducing interferon production, PROTEFLAZID® normalises local immune factors, including secretory IgA, lysozyme, and the C₃-component of complement. This results in the restoration of the barrier function of the vaginal and cervical mucosa. The

drug also helps correct vaginal dysbiosis, restores the physiological vaginal microenvironment, and promotes the regeneration of the cervical epithelium. These effects are particularly important for both the treatment and prevention of disease recurrence, as HPV entry into basal epithelial cells – the primary site of infection – requires a compromised epithelial barrier integrity and function.

PROTEFLAZID® also targets a key mechanism of HPV progression – impaired apoptosis. The drug enhances pro-apoptotic factors and activates caspase-9, thereby promoting the clearance of infected cells and supporting the primary prevention of chronic conditions associated with latent viral infections.

Thus, a direct antiviral effect against HPV, targeted influence on key mechanisms involved in HPV infection progression that lead to neoplastic transformation of the cervical epithelium, and suitability for prolonged use without inducing immune tolerance, support considering PROTEFLAZID® use in patients with mild to moderate CIN as an ethiopathogenetic therapy for HPV infection and as a means of secondary prevention of cervical cancer.

Currently, PROTEFLAZID® is available in two dosage forms: oral drops and vaginal suppositories. Both formulations may be used concurrently to provide systemic and local therapy, thereby enhancing the overall efficacy of HPV infection management.

Study Objective – to compare the efficacy of PROTEFLAZID® monotherapy in the treatment of HPV-induced CIN 1 and CIN 2 when administered systemically, locally, or as a combined systemic and local therapy.

Materials and Methods

The study included 90 women of reproductive age diagnosed with cervical intraepithelial neoplasia of varying degrees (CIN 1–2) caused by HPV infection. All patients were examined and treated at the Kyiv Municipal Center for Reproductive and Perinatal Medicine and were assigned to three groups.

Group I included 30 patients (33.33%) treated with PROTEFLAZID® oral drops, administered at a dose of 15 drops twice daily continuously for 3 months.

Group II included 30 patients (33.33%) who received local therapy with PROTEFLAZID® vaginal suppositories. The treatment regimen consisted of one suppository administered twice daily for 14 days each month during the intermenstrual period, with a total treatment duration of 3 months. Treatment commenced immediately after menstruation and was paused during menstruation.

Group III included 30 patients (33.33%) who received concurrently PROTEFLAZID® vaginal suppositories (one suppository administered twice daily for 14 days each month during the intermenstrual period, with a total treatment duration of 3 months), and PROTEFLAZID® oral drops (15 drops twice daily continuously for 3 months).

Considering that the transit time of an HPV-infected epithelial cell from the basal layer to the superficial layer may take up to three months, a prolonged treatment regimen of three months was selected to achieve viral clearance and prevent HPV infection recurrence.

Assessment of patients was conducted according to the protocols approved by the Ministry of Health of Ukraine and included evaluation of medical history and data on social status, general clinical tests and routine gynaecological examination with simultaneous collection of samples from the exocervix and endocervix for bacterioscopic and cervical cytological analysis (polychromatic Papanicolaou staining and monochromatic Pappenheim and

Giemsa staining). HPV was detected with polymerase chain reaction (PCR). The tests also included a semi-quantitative assessment of HPV DNA and evaluation of vaginal microbiota. Comprehensive diagnosis of vaginal and cervical infections was based on both bacterioscopic and bacteriological methods. Colposcopy, basic and advanced, was performed using 3% acetic acid and Lugol’s iodine solution to detect cervical epithelial abnormalities. In all patients, the diagnosis was confirmed by cytological findings. Additionally, pelvic ultrasound was performed to identify any concomitant gynaecological pathology.

Patients with inflammation and abnormal vaginal microbiota were excluded from the study.

The treatment efficacy and response were assessed based on the condition of the cervical epithelium and cervical canal as determined by cytological examination, HPV viral load, severity of colposcopic/colpocervicoscopic findings indicative of HPV infection, and the degree of epithelial dysplastic changes after completion of the treatment course in line with the proposed regimens.

Drug safety was evaluated based on monitoring data, frequency and types of adverse reactions, laboratory test results, and patients’ subjective health status.

Results and Discussion

All women enrolled in the study formed a representative sample in terms of age, medical history, and existing gynaecological pathology. The mean age of patients was 25 ± 0.5 years in Group I, 26 ± 0.5 years in Group II, and 25 ± 0.5 years in Group III, with no significant differences in the groups.

Based on medical history, patients aged 20–26 years presented with concomitant gynaecological pathologies, such as dyshormonal menstrual disorders (including anovulation, hyperpolymenorrhea, luteal phase insufficiency and dysmenorrhoea); patients aged 27–31 years exhibited ovarian cysts and infertility, and patients aged 32 years and older had endometriosis and uterine leiomyoma (Table 1). Regarding the parity of childbirth, it is important to note that 85% of patients were nulliparous.

Table 1: Correlation of patients’ age and comorbidities, n=90 (%)

Age, years	Concomitant gynaecological pathology					
	Dysmenorrhoea	Ovarian cysts	Menstrual disorders	Infertility	Uterine leiomyoma	Endometriosis
20–26	8 (21,6)	4 (10,8)	14 (37,8)	-	-	4 (10,8)
27–31	2 (6,3)	7 (21,9)	2 (6,3)	6 (18,8)	2 (6,3)	4 (12,5)
≥ 32	1 (4,8)	2 (9,5)	1 (4,8)	1 (4,8)	10 (47,6)	8 (38,1)

The primary complaints reported by patients across all groups were similar and included intermittent “contact” bleeding from the genital tract during sexual intercourse or cytological sampling, and discomfort in the external genital area.

Cytologically, all patients showed specific signs of HPV infection: clearly visualised perinuclear necrosis (koilocytic zone), presence of binucleated koilocytes, mild dyskaryosis and nonspecific signs of HPV – uneven cytoplasmic staining, increased squamous epithelial cell size. 57 (63.5%) of patients had mild CIN. In 33 (36.7%) of the examined patients from Groups I, II and III, cytological findings revealed the presence of moderate CIN.

Table 2: Comparative characteristics of the cervical epithelium according to cytological data (pre and post treatment)

Parameter	Group					
	Pre treatment		Post treatment		Regression in the group	
	n	%	n	%	n	%
Group I	30	100,0	30	100,0	18	60,0
Type 1	0	0,0	12	40,0		
CIN 1	18	60,0	12	40,0	12	66,7
CIN 2	12	40,0	6	20,0	6	50,0
Group II	30	100,0	30	100,0	19	63,3
Type 1	0	0,0	14	46,7		
CIN 1	20	66,7	11	36,7	14	70,0
CIN 2	10	33,3	5	16,7	5	50,0
Group III	30	100,0	30	100,0	25	83,3
Type 1	0	0,0	18	60,0		
CIN 1	19	63,3	8	26,7	18	94,7
CIN 2	11	36,7	4	13,3	7	63,6

The cytological findings on the cervical epithelium before treatment are summarised in Table 2. Mild cervical intraepithelial neoplasia was identified in 18 patients (60.0%) in Group I, 20 patients (66.7%) in Group II, and 19 patients (63.3%) in Group III. Moderate CIN was detected in 12 women (40.0%) in Group I, 10 (33.3%) in Group II, and 11 (36.7%) in Group III.

All patients were diagnosed with endometrial dysplasia based on the results of basic and advanced colposcopy. The following findings prevailed on colposcopy: punctation in 41 (45.6%), acetowhite epithelium in 27 (30.0%), leukoplakia in 4 (4.5%), and mosaic in 8 (8.9%) cases.

Table 3: Comparative characteristics of HPV DNA presence in the cervical epithelium according to PCR (pre and post treatment)

Parameter	Group					
	Pre treatment		Post treatment			
	Present DNA of HPV		Present DNA of HPV		Absent DNA of HPV	
	n	%	n	%	n	%
Group I	30	100	8	26,7	22	73,3
CIN 1	18	100	3	16,7	15	83,3
CIN 2	12	100	5	41,7	7	58,3
Group II	30	100	7	23,3	23	76,7
CIN 1	20	100	3	15,0	17	85,0
CIN 2	10	100	4	40,0	6	60,0
Group III	30	100	3	10,0	27	90,0
CIN 1	19	100	1	5,3	18	94,7
CIN 2	11	100	2	18,2	9	81,8

In addition, PCR testing was done in all women to determine the presence and type of HPV before treatment and 2 months after the end of the treatment course (Table 3). All patients were HPV positive before treatment. According to the tests, patients with CIN 1 in all groups were infected with one type of high oncogenic HPV strain, and patients with CIN 2 had a combination of several HPV types (high oncogenic strain and low oncogenic strain).

Systemic monotherapy with PROTEFLAZID® (drops – Group I) contributed to the normalisation of the colposcopy findings and the transition of CIN 1 to Pap Class 1 in 12 (66.7%) of 18 women, and the regression of CIN 2 to CIN 1 in 6 (50%) of 12 women (see Table 2). It should be noted that the highest efficiency was observed in patients with a small area of cervical lesions. In general, CIN regression in Group I occurred in 18 (60%) patients. Repeat PCR test (see Table 3) showed HPV presence in 22 (73.3%) women in Group I. The best rate of virus elimination was seen in women with CIN 1 – 15 (83.3%) cases versus 7 (58.3%) in women with CIN 2.

The study showed that PROTEFLAZID® (suppositories) has a slightly higher efficacy compared to the drops when used for the treatment of mild to moderate cervical intraepithelial neoplasia associated with HPV. Topical monotherapy with PROTEFLAZID® (suppositories, Group II) resulted in the regression of neoplasia in a total of 19 (63.3%) women, regression of CIN 2 to CIN 1 in 5 (50%) of 10 women and transition of CIN 1 to normal cytological findings in 14 (70%) of 20 women (see Table 2). On repeat PCR test, HPV was not detected in 17 (85%) women with CIN 1, and 6 (60%) women with CIN 2; in Group II, HPV elimination was seen in 23 (76.7%) patients (see Table 3).

In the study, the highest efficacy of PROTEFLAZID® therapy in women with CIN 1-2 was observed in Group III, where patients were treated with PROTEFLAZID® suppositories in combination with PROTEFLAZID® drops (using the regimen described above). The patients with CIN 1-2 exhibited improvement in the cytological (reduction of leucocytosis, koilocytosis, number of cells with dyskeratosis) and colposcopy findings. Namely, regression of neoplasia was seen in 25 women (83.3%) in Group III; transition of CIN 2 to CIN 1 in 7 (63.6%) of 11 patients and normalisation of the cytological findings in 18 (94.7%) of 19 patients with CIN 1 (see Table 2). The repeat PCR test results showed that 27 women (90.0%) were negative for HPV: 18 (94.7%) with CIN 1 and 9 (81.8%) with CIN 2 (see Table 3).

Thus, the combined use of PROTEFLAZID® drops (administered systemically per os) and PROTEFLAZID® suppositories (administered topically) helps to reduce the lesion area and the severity of the pathological process, enabling conservative treatment in 90% of patients with this condition.

HPV infection begins in the basal layer and spreads to the surface of the cervix, i.e. the virus is present in all layers of the epithelium. Therefore, to ensure HPV elimination, it is necessary to create high concentrations of the antiviral drug in all layers of the epithelium.

PROTEFLAZID® suppositories, acting locally, provide maximum concentrations of the active drug in the superficial layers of the cervical epithelium, and PROTEFLAZID® drops enter the basal layers of the epithelium in maximum amounts from the blood vessels of the underlying tissues.

Thus, combined systemic and topical administration of PROTEFLAZID® (drops and suppositories) provides a dual direct effect on the virus – systemic and local – which increases the effectiveness of antiviral therapy.

No serious or unexpected adverse reactions or laboratory abnormalities were observed during the treatment, allowing the therapy to be considered well tolerated.

Conclusions

1. The treatment of CIN 1 and CIN 2 associated with HPV infection using PROTEFLAZID® is etiologically and pathogenetically justified due to its direct antiviral activity against HPV and its effects on the transformation of cells affected by the virus.

2. Systemic administration of PROTEFLAZID® (drops) per os in the treatment of mild to moderate neoplasia leads to improvement of the cervical epithelium in 60% of patients and eradication of HPV in 73.3% of patients.

3. Topical administration of PROTEFLAZID® (suppositories) in CIN 1–2 normalises the cytological findings or leads to the transition of CIN 2 to CIN 1 in 63.3% of women; HPV DNA was not detected after treatment in 76.7% of women.

4. Combined topical and systemic administration of PROTEFLAZID® suppositories and PROTEFLAZID® drops over 3 months for the treatment of CIN 1 and CIN 2 associated with HPV infection resulted in the regression of CIN 1 to normal and CIN 2 to CIN 1 in 83.3% of patients; HPV DNA was not detected after treatment in 90.0% of patients. Thus, this regimen provides the highest therapeutic efficacy.

5. PROTEFLAZID® can be recommended for clinical use as a safe and effective monotherapy for patients with CIN 1 and CIN 2 caused by HPV infection, particularly when administered in a combined systemic and topical regimen (suppositories + drops), which provides the highest therapeutic efficacy.

6. The proposed regimen of combined PROTEFLAZID® administration (suppositories + drops) appears to be a promising approach for the treatment of CIN 1 and CIN 2 in women of reproductive age, as it helps avoid cervical damage, prevents early and late complications associated with destructive methods of treatment and preserves reproductive function.

References:

1. Jemal A., Bray F., Center M. M., Ferlay J., Ward E., Forman D. / Global cancer statistics. // *C A Cancer J Clin.* 2011 Mar-Apr; 61 (2) : 69–90.
2. McCredie M. R., Sharples K. J., Paul C. et al. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. *Lancet Oncol.* 2008; 9 : 425–34.
3. Schiffman M., Castle P. E., Jeronimo J., Rodriguez A. C. and Wacholder S. Human papillomavirus and cervical cancer. *Lancet.* 2007; 370 : 890–907.
4. Snijders P. J., Steenbergen R. D., Heideman D. A., and Meijer C. J. HPV-mediated cervical carcinogenesis: concepts and clinical implications. *J. Pathol.* 2006; 208 : 152–64.
5. Патологія шийки і тіла матки: керівництво для лікарів / Під ред. Коханевич Є. В. – Ніжин: Гідромакс, 2009. – 350 с.
6. Папіломавірусна інфекція: актуальна проблема сучасної гінекології / Воробійова Л. І. // *Здоров'я жінки.* – 2015. – №3 (99). – С. 21–24.
7. Ефективність застосування та переносимість імуномодуляторів у жінок із папіломавірусною інфекцією, поєднаною з передпухлинними процесами шийки матки та уrogenітальною мікст-інфекцією / Суслікова Л. В., Борис О. М., Суменко В. В., Камінський А. В., Сербенюк А. В. // *Здоров'я жінки.* – 2009. – №4 (40). – С. 140–146.

8. Кисельова В. І., Крикунова Л. І., Любіна Л. В. Інфікування вірусом папіломи людини і прогноз РШМ // Питання онкології. – 2010. – №2. – С. 185–187.
9. Accuracy of liquid based versus conventional cytology: overall results of new technologies for cervical cancer screening: randomised controlled trial / Ronco G., Cuzick J., Pierotti P. [et al.] // BMJ. – 2007. – Vol. 335 (7609). – P. 28–38.
10. Oster A. G. Natural history of CIN: a critical review // In. J. Gynecol. Pathol. – 1993. – Vol. 12. – P. 186–192.
11. Updating the natural history of HPV and anogenital cancer. Chapter 5. / Moscicki A. B., Schiffman M., Kjaer S., Villa L. L. // Vaccine. – 2006. – Vol. 24, suppl. 3. – P. S42–51.
12. Мінкіна Г. Н., Манухін І. Б., Франк Г. А. Передрак шийки матки – М.: Аерографмедіа, 2001. – 112 с.
13. Chabner B. A., Lynch T. J., Longo D. L. Harrison's Manual of Oncology. – New York: McGraw-Hill Medical, 2011. – 656 p.
14. Питання і теми охорони здоров'я. Рак шийки матки [Електронний ресурс] / Всесвітня організація охорони здоров'я – Режим доступу: <http://www.euro.who.int/ru/healthtopics/noncommunicablediseases/cancer/news/news/2012/2/earlydetection-of-common-cancers/cervicalcancer>.
15. Інформаційний бюлетень №380, березень 2015 г. [Електронний ресурс] / Всесвітня організація охорони здоров'я – Режим доступу: <http://www.who.int/mediacentre/factsheets/fs380/ru/>
16. Інформаційна записка ВООЗ. Комплексна профілактика раку шийки матки та боротьба з ним – здорове майбутнє для дівчаток і жінок / Всесвітня організація охорони здоров'я – L'IV Com Sàrl, Villarssous-Yens, Switzerland, 2013. – 13 с.
17. Рак в Україні 2015–2016. Захворюваність, смертність, показники діяльності онкологічної служби : Бюл. Нац. канцер-реєстру України. – Київ, 2017. – №18. – 127 с.
18. Манжура Є. П. Цервікальна інтраепітеліальна неоплазія (CIN). Сучасні підходи до діагностики, лікування та реабілітації // Здоров'я жінки. – 2016. – №5 (111). – С. 19–25.
19. Кишакевич І. Т. Сучасні аспекти лікування доброякісних процесів шийки матки, асоційованих з папіломавірусною інфекцією // Педіатрія, акушерство та гінекологія. – 2004. – №2. – С. 116–118.
20. Моделювання папіломавірусної інфекції *in vitro* та вивчення впливу Протефлазиду на репродукцію вірусу папіломи / Рибалко С. Л., Порва Ю. І., Старосила Д. Б., Дядюн С. Т., Руденко А. В. // Матеріали симпозіуму (7–9 червня 2011 р.). – СПб., 2011. – С. 29–31.
21. Роговська С. І. Папіломавірусна інфекція у жінок та патологія шийки матки. – М., 2008. – С. 14.
22. Кутушова Г. Ф. та співавт. Практична онкологія – 2009. – Т. 10, №2. – С. 63.
23. Суханова А. А. Особливості ВПЛІ-інфекції у жінок України // Акушерство. Гінекологія. Генетика. – 2016. – Т. 2, №2. – С. 12–22

Reference: Kaminskiy V. V., Sukhanova A. A., Shalko M. N., Sumenko V. V., Lavrenjuk Y. V. (2019). *Comparative Effectiveness of Different PROTEFLAZID® Formulations in the Treatment of Mild to Moderate Cervical Intraepithelial Neoplasia (CIN) Associated with Genital HPV Infection. Woman's Health, 5 (137), 48–54.*

3.4. THE USE OF FLAVONOID GLYCOSIDES IN THE TREATMENT OF GENITAL HPV INFECTION IN WOMEN

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Abstract

Objective – to assess the clinical efficacy of flavonoid glycosides derivatives in the treatment of benign and precancerous cervical disease. The study was conducted at the Odessa Regional Clinic Hospita in 2010-2013. The study involved 80 women of reproductive age (mean age $28,4 \pm 0,2$ years) with verified epithelial cervical dysplasia mild to moderate (index group) and 30 healthy women surveyed in order of medical examination. Human papilloma virus was quantified in fixed preparations using real-time PCR according to standard procedure. For immunohistochemical determination CD1a murine monoclonal antibody in a dilution of 1: 25 was used. All patients had dysplasia of cervical squamous epithelium, including in the stage of CIN I in 27 (54.0%) cases, CIN II – 15 (30.0%) cases, CIN III – 8 (16.0%) cases. Some patients had dysplasia and comorbidied diseases of the genitals. Human papillomavirus in 84.5% of cases belong to the group A9 (types 16, 31, 33, 35, 52, 58). Application in the complex treatment proteflazid 30% reduced viral load in genital human papillomavirus infection and helped to increase the number of antigen-presenting cells. Standard therapy was less effective by virostatic and anti-relapse effect than the combined method of treatment with the use of proteflazid.

Key words: flavonoid glycosides, benign disease of uterine cervix, precancer disease of uterine cervix.

Introduction

Cervical cancer (CC) is the second most common malignancy among women in developed countries, following breast cancer [1]. According to the National Cancer Registry of Ukraine, the incidence of cervical cancer in 2011 was 15.4 per 100,000, with a mortality rate of 5.5 per 100,000 population [2].

Human papillomavirus (HPV) plays a leading role in the etiopathogenesis of cervical precancerous lesions and cervical cancer [3–5]. Given that epithelial dysplasia of varying severity is found adjacent to areas of carcinoma in situ in approximately 85% of cases, early diagnosis and treatment of such conditions are critical [6].

The development of epithelial dysplasia is a dynamic process influenced by a range of clinical and biological factors, including the immune system function, the oncogenic potential of the HPV type, the patient's age, infection duration, and co-infections. According to the literature, spontaneous disease regression may occur in nearly 57% of patients with mild, 44% with moderate, and 32% with severe dysplasia [1, 3, 5]. However, the underlying mechanisms of this phenomenon remain unclear.

In recent years, it has been demonstrated that the process of HPV infection includes two distinct stages: the productive phase, during which the viral DNA exists in an episomal (non-integrated) form, and the integrative phase, during which the viral DNA becomes incorporated into

the genome of infected host cells. The productive phase is reversible, and spontaneous remission occurs in many infected individuals. By contrast, the integrative phase represents a critical step toward malignant transformation and is frequently associated with the development of carcinoma [1, 3, 4, 6]. The risk of malignant transformation increases significantly when HPV interacts with other carcinogenic or infectious cofactors. The incorporation of the virus into the genome is accompanied by upregulated synthesis of the E6 and E7 proteins, which initiate a cascade of reactions leading to uncontrolled cellular proliferation. This process is counteracted by the p16INK4a protein, the synthesis of which markedly increases in the case of the integrative HPV infection. In addition, according to some researchers, one of the mechanisms of malignisation of HPV-infected cells involves viral-induced alterations of cellular metabolism. Specifically, HPV may shift oestradiol metabolism towards preferential synthesis of 16 α -hydroxyestrone (16 α -OH), which is known to directly upregulate the expression of the E7 gene initiating neoplastic transformation [5, 6]. The resulting E7 oncoprotein promotes pathological cell proliferation while simultaneously blocking the development of immune protection – an effect with specific features characteristic of HPV infection [7, 8]. Similar to other chronic viral infections, persistent HPV infection often leads to immunodeficiency, classified as secondary immunodeficiencies, which result from dysfunction in various components of the immune system [8]. It should be noted that HPV cannot infect antigen-presenting dendritic cells, which explains the absence of a direct pathway for immune activation in HPV infection. Persistent HPV infection is facilitated by a well-documented ability of the virus to evade systemic immune surveillance. Although HPV primarily infects basal epithelial cells, viral replication and assembly of viral particles occur in differentiated cells of the superficial epithelial layer, which subsequently undergo apoptosis. This process typically proceeds without inflammation and is largely ignored by the immune system [8, 9]. However, effector immune cells secrete a range of cytokines (including α -, β -, and γ -interferons), which are known to suppress transcription of the E6 and E7 genes in HPV types 16, 18, and 33 within virally transformed cells. Cellular immune dysregulation in HPV infection is supported by evidence such as the observed combination of spontaneous regression of HPV-induced hyperplastic lesions and infiltration of the surrounding tissues with lymphocytes and macrophages. Moreover, some studies suggest that the elimination of HPV from the host may be linked to the emergence of T-helper responses targeting the C-terminal domain of the viral E2 protein [8].

One of the most promising approaches in the treatment and secondary prevention of viral infections is the use of flavonoid glycosides, which are potent immunomodulators and can influence apoptosis. Importantly, these compounds also have a direct effect on the activity of virus-specific enzymes, including thymidine kinase, DNA polymerase, reverse transcriptase [10, 11], and DNA-dependent RNA polymerase, resulting in a pronounced virostatic effect [10]. However, to date, the efficacy of flavonoid glycoside derivatives in the management of HPV-induced hyperplastic lesions has not been sufficiently studied.

The **Study Objective** is to evaluate the clinical efficacy of flavonoid glycoside derivatives in the treatment of benign and precancerous cervical diseases.

Materials and Methods

The study was conducted at the Regional Clinical Hospital (Odesa, UKRAINE) between 2010 and 2013. It involved 80 women of reproductive age with confirmed mild to moderate cervical epithelial dysplasia (treatment group) and 30 clinically healthy women after routine screening (control group).

All participants enrolled in the study underwent comprehensive evaluation in line with current clinical protocols as outlined in the Orders by the Ministry of Health of Ukraine No.582 dated 15.12.2003, No.676 dated 31.12.2004, and No.624 dated 03.11.2008 [12].

HPV in fixed tissue samples was quantitatively assessed using real-time PCR according to a standardised protocol.

For immunohistochemical detection of CD1a (a marker for dendritic cells), mouse monoclonal antibodies to CD1a (Clone O10, Cat. No.: 1590, Immunotech, France) were used at a dilution of 1:25. Microphotographs of the obtained specimens were analysed using ImageJ 1.48d software (NIH, USA). The CD207 antigen (type II transmembrane lectin, langerin) – an endocytic receptor with mannose specificity, was quantitatively detected by Western blotting (Langerin Antibody (E-17), Santa Cruz Biotechnology, USA). Cruz Marker™ anti-mouse IgG-HRP antibodies (sc-2031, dilution range: 1:2000–1:5000) were used as secondary antibodies. Additional reagents included Cruz Marker™ Molecular Weight Standards (sc-2035), TBS Blotto A Blocking Reagent (sc-2333), and Western Blotting Luminol Reagent (sc-2048) (USA).

Patients in the treatment group were randomized into two clinical subgroups depending on the intervention type. Group I (n=40) received standard pharmacological therapy, while Group II (n=40) was treated with the direct antiviral agent with immunomodulatory properties, PROTEFLAZID®, administered orally as drops for 4–8 weeks, and locally (vaginal tampons) for 14 days twice daily, with a 10-day interval, using a 1:4 dilution of PROTEFLAZID® in physiological saline solution.

Statistical analysis was performed using analysis of variance (ANOVA) and correlation methods with the Statistica 10.0 software package (StatSoft Inc., USA) [13].

Results

The mean age of the patients was 28.4 ± 0.2 years. All patients were diagnosed with cervical squamous epithelial dysplasia: 27 women (54.0%) had CIN 1, 15 women (30.0%) had CIN 2, and 8 women (16.0%) had CIN 3. Some patients also presented with various inflammatory genital conditions alongside dysplasia: vulvovaginitis (6.0%), bacterial vaginosis (12.0%), cervicitis (6.0%), candidiasis (4.0%), and chronic endometritis (10.0%).

Among extragenital pathologies, the most common were ENT disorders, including nasopharyngitis (6.0%), tonsillitis (4.0%), maxillary sinusitis (2.0%), upper respiratory tract infections (bronchitis, 4.0%), urinary system disorders such as chronic pyelonephritis (4.0%), glomerulonephritis (2.0%), cystitis (4.0%), gastrointestinal diseases including gastritis (4.0%), gastroduodenitis (4.0%), biliary dyskinesia (2.0%), neurocirculatory dystonia (10.0%), and allergic diseases such as pollinosis and atopic dermatitis. Fourteen women (28.0%) were classified as patients with recurrent respiratory infections (experiencing four or more episodes of acute respiratory infections per year). HPV infection was confirmed in 82.0% of the patients. HPV viral load ranged from 1,000 to 6,628 copies per reaction. In most patients (78.0%) the viral load did not exceed 5 lg copies per 105 cells. In the control group, asymptomatic HPV-induced hyperplastic lesions were observed in 13.3% of patients, with viral loads at the threshold of clinical significance (2–3 lg copies per 105 cells). In the vast majority of cases (85.4%), HPV types from the A9 species group were detected, which includes types 16, 31, 33, 35, 52, and 58. In one patient, high titers (6.5 log copies per 105 cells) of A5/A6 HPV species were identified (types 51 and 56). No cases of HPV A7 species (types 18, 39, 45, 59) were detected.

All patients with cervical neoplasia showed a reduction in the population of CD1a+ and CD83+ cells in the cervical epithelium. Specifically, the mean number of immature CD1a+ dendritic cells in patients with cervical intraepithelial neoplasia was 2.7 ± 0.1 per high-power field (HPF), and that

of mature CD83+ dendritic cells was 0.02 ± 0.002 per HPF, whereas in healthy women these values were 3.1 ± 0.1 CD1a+ cells per HPF and 0.04 ± 0.003 CD83+ cells per HPF, respectively.

Further studies demonstrated that standard therapy was inferior in terms of virostatic and anti-relapse effects compared to treatment with PROTEFLAZID®. In particular, HPV viral load decreased by $3.0 \lg D_{50}$ after treatment (see Fig. 1).

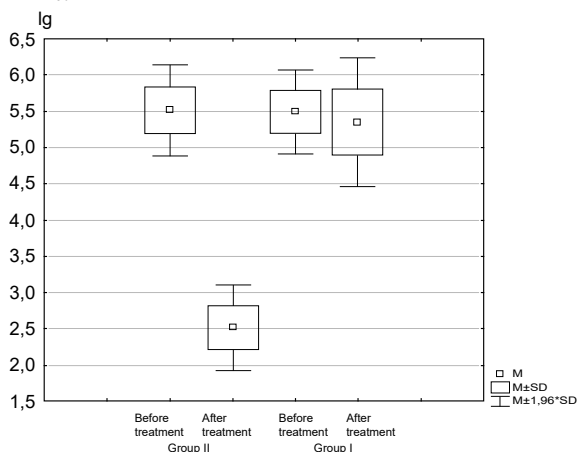


Fig. 1. Changes in HPV viral load in the study groups

As shown in Fig. 1, the viral load in patients treated with the standard regimen remained practically unchanged, accompanied by a significant increase in the variability of the indicator. In contrast, Group II demonstrated a significant reduction in viral load by $3.0 \lg D_{50}$.

At the same time, the number of dendritic cells increased, indicating a positive effect of flavonoid glycosides on cellular immunity. Specifically, following treatment, there was a marked increase in the number of immature dendritic cells, identified by the CD1a marker (see Fig. 2).

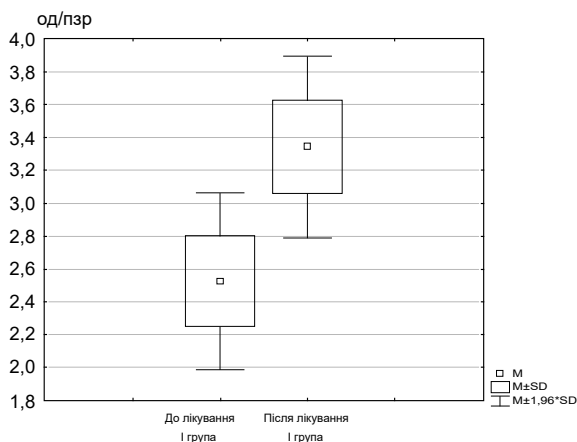


Fig. 2. Changes in the population of immature dendritic cells

A similar trend was observed in the number of mature dendritic cells, specifically CD83+ cells (Fig. 3.)

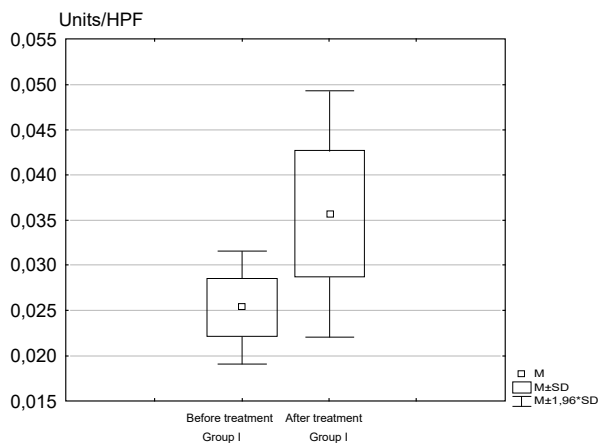


Fig. 3. Changes in the population of mature dendritic cells

Clinical experience with both systemic and intravaginal administration of flavonoid glycoside derivatives for the treatment of benign and precancerous cervical pathology demonstrates substantial therapeutic efficacy of this drug. Compared to standard therapy, the combination regimen with PROTEFLAZID® exhibits superior virostatic properties and reduces the risk of recurrence. Considering that PROTEFLAZID® reduces HPV viral load by 3.0 lg D₅₀ and concurrently increases the number of antigen-presenting cells, its incorporation into combination therapy for all patients with HPV infection appears clinically justified and advisable.

Conclusions

1. PROTEFLAZID® reduces the viral load in HPV-induced hyperplastic lesions by 3.0 lg D₅₀.

2. PROTEFLAZID® increases the number of antigen-presenting cells.

Standard therapy is inferior to combined treatment with PROTEFLAZID® in terms of virostatic and relapse-preventive effects.

References:

1. Mild obesity, physical activity, calorie intake, and the risks of cervical intraepithelial neoplasia and cervical cancer. / Lee J. K., So K. A., Piyathilake C. J., Kim M. K. // PLoS One. 2013 Jun 12;8(6):e6655
2. Comprehensive control of human papillomavirus infections and related diseases. / Bosch F. X., Broker T. R., Forman D. [et al.] // Vaccine. – 2013 – Vol. 31 Suppl 6: G1–G31
3. The Globocan project. Estimated Cancer Incidence, Mortality & Prevalence Worldwide in 2012. Электронний ресурс. Режим доступу: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx
4. Національний канцер-реєстр України. Рак шийки матки. Електронний ресурс. Режим доступу: http://www.ncru.inf.ua/publications/BULL_14/PDF/16-shm.pdf

5. Михайлович Ю. Й. Скринінг раку в Україні: коли теорія відповідає реальності. Соціально-економічне обґрунтування популяційного скринінгу раку шийки матки. / Михайлович Ю. Й., Журбенко А. В. // Клінічна онкологія. – 2011 – №3 – С. 6–11
6. Developing role of HPV in cervical cancer prevention. / Kitchener H. C., Denton K., Soldan K., Crosbie E. J. // BMJ. – 2013 – Vol. 347 – f4781
7. Шелестова Л. П. Фонові й передракові захворювання жіночих статевих органів. / Шелестова Л. П. // Медико-соціальні проблеми сім'ї. – 2012 – т. 17, №3–4 – С. 120–123
8. Schiffman M. Clinical practice. Cervical-cancer screening with human papillomavirus and cytologic cotesting. / Schiffman M., Solomon D. // N Engl J Med. – 2013 – Vol. 369(24) – P. 2324–2331
9. Risk factors and clinical aspects of recurrent invasive cervical carcinoma. / Ghaemmaghami F., Saleh-Gargari S., Sahebdel B. [et al.] // J Obstet Gynaecol India. – 2012 – Vol. 62(6) – P. 674–648

3.5. COMPARATIVE EFFICACY AND SAFETY OF PROTEFLAZID® SUPPOSITORIES AND DROPS IN PATIENTS WITH CERVICAL EPITHELIAL DYSPLASIA CAUSED BY HPV INFECTION

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Abstract

The paper presents data from a phase II clinical trial comparing the efficacy and tolerability of PROTEFLAZID® suppositories and drops in patients with cervical dysplasia caused by HPV infection. The trial showed that the high therapeutic effectiveness of suppositories PROTEFLAZID® is comparable to that of drops PROTEFLAZID®. Suppositories PROTEFLAZID® can be recommended as an effective and safe agent for the treatment of this patient population.

Key words: PROTEFLAZID®, suppositories, drops, human papillomavirus infection, cervical epithelial dysplasia.

Introduction

In obstetric, gynaecological, and urological practice, diseases caused by human papillomavirus (HPV) represent a significant public health concern. The diversity of clinical presentations, diagnostic approaches to various forms of HPV infection, and patient management strategies remain controversial and lack unified standards. This is primarily due to the absence of pharmacological agents with direct antiviral activity against HPV. In recent years, there has been a notable increase in the use of unjustified or overtly aggressive treatment approaches in HPV-positive women, which frequently leads to long-term complications and high recurrence rates, particularly during pregnancy (Voloshyna N. M., 2006; 2007; Voloshyna N. M. et al., 2007).

The standard treatment for clinically manifest HPV infection typically involves surgical methods such as electrocoagulation, cryodestruction, and similar techniques. Additionally, therapeutic approaches include agents inducing chemical necrosis of exophytic HPV lesions.

To prevent disease recurrence, current strategies employ recombinant interferons, inducers of endogenous interferon production, and topical antiviral preparations. However, these methods do not ensure a sustained clinical effect in all patients (Radionov V. H. et al., 2002; Voloshyna N. M., 2007).

To suppress HPV replication, it is necessary to use antiviral agents. One such agent is PROTEFLAZID® in suppository form – an antiviral drug with immunotropic properties. Its active ingredients (flavonoids) inhibit the synthesis of viral DNA and RNA in infected cells by suppressing the activity of virus-specific RNA and DNA polymerases, thymidine kinase, and reverse transcriptase. The drug also promotes the synthesis of endogenous α - and γ -interferons to physiologically active levels (without inducing refractoriness), thereby enhancing local nonspecific resistance to viral and bacterial infections. Clinical trials have shown that PROTEFLAZID® suppositories help restore the protective function of the vaginal and cervical mucosa by normalising local immunity factors (secretory IgA – sIgA, lysozyme, and complement C₃). The active substance has specific antiviral activity and inhibits HPV replication in experimental models *in vitro* involving oncogenic HPV strains. Cytological studies have shown that the drug suppresses the proliferative and cytopathic effects of HPV on host cells.

In genital herpes, PROTEFLAZID® (suppositories) prevents new lesions, reduces the risk of dissemination and visceral complications, and accelerates the healing of affected areas. In vaginosis, vaginitis, and inflammatory diseases of the cervix, the drug contributes to the restoration of local immunity and facilitates more rapid and effective pathogen elimination. PROTEFLAZID® (suppositories) exhibits antioxidant properties by inhibiting free radical processes and thereby preventing the accumulation of lipid peroxidation products, which enhances the cellular antioxidant status. The drug also acts as an apoptosis modulator, amplifying the effects of pro-apoptotic factors. In particular, by activating caspase-9, it promotes faster elimination of virus-infected cells and contributes to the primary prevention of chronic conditions associated with latent viral infections.

The efficacy and tolerability of PROTEFLAZID® suppositories were evaluated in 30 patients with genital herpes in clinical remission during a phase I clinical trial conducted by the Department for Female Reproductive Function Rehabilitation at the SI “Institute of Paediatrics, Obstetrics and Gynaecology of the NAMS of Ukraine.” Treatment was associated with a reduction in virus-specific IgG and sIgA, along with an increase in lysozyme and complement component C₃ levels in cervical mucus. These changes indicated an enhancement of both systemic and local immune resistance. The drug was well tolerated, with no serious adverse effects and no clinically significant alterations in laboratory parameters or physical examination findings.

Study Objective

To conduct a comparative evaluation of the efficacy and tolerability of PROTEFLAZID® suppositories [hereinafter referred to as the “test drug (TD)”] and PROTEFLAZID® oral drops [hereinafter referred to as the “control drug (CD)”] in patients with cervical epithelial dysplasia (CED) caused by HPV infection.

Study Design and Methods

A phase II open-label, randomised, controlled clinical trial in parallel groups included 76 women aged 18–50 years diagnosed with cervical epithelial dysplasia caused by HPV

infection. All participants received inpatient treatment at the Department of Reproductive and Endocrine Gynaecology, Kyiv Municipal Center for Reproductive and Perinatal Medicine.

Clinical assessment for all participants included physical examination, complete blood count and urinalysis, blood chemistry tests, bacteriological and bacterioscopic analysis of cervical canal (CC), urethral and vaginal samples, HPV DNA detection and genotyping in smears from CC/cervix by polymerase chain reaction, cytological examination of smears from the cervix and CC using the Papanicolaou test with evaluation of koilocytosis, measurement of local immunity parameters (sIgA, lysozyme, C₃ complement) at the end of the treatment, and colposcervicospscopy with assessment of CED features.

The cytological findings in patients' smears corresponded to CIN (cervical intraepithelial neoplasia) grade 1–2, or mild to moderate cervical epithelial dysplasia (CED). All participants presented with high-risk oncogenic HPV DNA detected in smears from the epithelium of the CC/cervix by polymerase chain reaction, as well as koilocytosis. Women of reproductive age were tested negative for pregnancy. Participants were instructed to avoid sexual intercourse during the study period. All patients provided their written informed consent prior to enrolment.

The participants were divided into two statistically homogeneous groups (n=38 each), comparable in terms of age, gynaecological history, cytological findings from cervical and endocervical smears, local immunity markers and colposcervicospscopic findings. No statistically significant differences were observed between the groups in haemodynamic parameters or body temperature.

After normalisation of the vaginal microbiota (if required), patients in the first (treatment) group used the test drug (TD) by inserting it deep into the vagina after hygiene procedures, twice a day; treatment was recommended to start immediately after menstruation. Participants in the second (control) group used the control drug (CD) in the form of vaginal tampons soaked with a drug solution (3.0 ml [72–75 drops] of the product diluted in 20.0 ml of normal saline); the exposure time was 30–40 minutes, twice a day.

The duration of treatment was 14 days, with an 8-week follow-up period during which the patients were asked to report any possible side effects and cases of disease recurrence.

The primary efficacy endpoint was the condition of the epithelium of the cervix and CC based on cytological findings at the end of the treatment. Secondary endpoints included the condition of the epithelium of the cervix and CC based on cytology at the end of the follow-up period, the HPV viral load at the end of treatment and follow-up period, the severity of colposcopic/colposcervicospscopic signs of HPV at the end of treatment and follow-up period, and local immunity markers at the end of treatment (sIgA, lysozyme, and complement component C₃).

Results and Discussion

All participants completed the full 14-day treatment course.

The changes in HPV DNA viral load between the groups are presented in Fig. 1, and changes in local immunity parameters are given in Figs. 2–4. Based on the analysis, it was concluded that HPV DNA viral load significantly reduced and local immunity significantly improved in both groups.

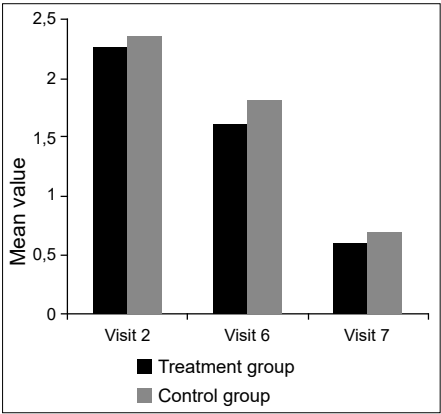


Fig. 1. Changes in HPV DNA viral load across groups

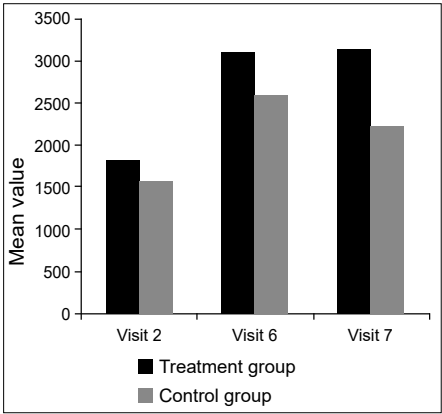


Fig. 2. Changes in sIgA levels across groups

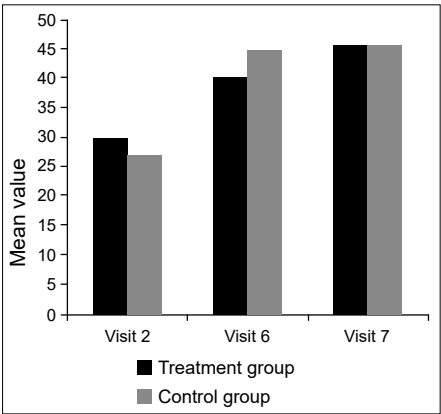


Fig. 3. Changes in lysozyme levels across groups

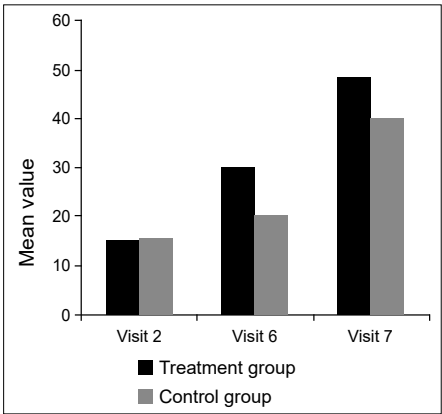


Fig. 4. Changes in complement component C₁ levels across groups

Significant improvement in colposcervicoscopy findings was observed in the majority of patients in both the treatment and control groups. The therapy was found to be effective in 38 (100.0%) patients in the treatment group and 35 (92.11%) in the control group. Based on the data presented in Table 1, it was concluded that there was no statistically significant difference between the groups in terms of treatment efficacy.

The conclusion about the non-inferiority of the TD's efficacy compared to that of the CD in the analysed population of patients was based on the confidence interval (CI) approach (Table 2). Since the lower limit of the 95% CI (-9.18%) is above the predefined non-inferiority margin (-20%), it was concluded that the TD is non-inferior to the CD in terms of efficacy.

No significant differences were observed between the groups in the assessment points related to the changes in HPV DNA viral load and local immunity indicators throughout the study period.

Table 1. Distribution of patients by efficacy endpoints

Drug efficacy	Group			
	Treatment (n=38)		Control (n=38)	
	Rate	%	Rate	%
Effective	38	100,0	35	92,11
Non-effective	-	-	3	7,89

Table 2. 95% CI limits for difference between proportions of positive outcomes

Statistical parameter	Value
Probability of type I error, α	0.025
Percentage point of standard normal distribution for α	1.96
Non-inferiority margin (δ), %	20
Proportion of positive outcomes in the treatment group, %	100.00
Treatment group size	38
Proportion of positive outcomes in the control group, %	92.11
Control group size	38
Proportion difference, %	7.89
Lower limit of 95% CI	-9.18
Upper limit of 95% CI	9.18

Haemodynamic parameters and body temperature in both groups did not change significantly during the study.

Statistical analysis showed that in most cases, there were no statistically significant differences in the majority of parameters of complete blood count, blood chemistry, and urinalysis before and after the treatment in both groups.

No serious adverse reactions or cases of early withdrawal from the study due to undesirable effects were reported.

Conclusions

1. The test drug is highly effective and is non-inferior to the control drug in terms of therapeutic efficacy in patients with cervical epithelial dysplasia (CED) caused by HPV infection.

2. By the end of the treatment course with the test drug, cytological normalisation or regression of cervical intraepithelial neoplasia from CIN-2 (moderate CED) to CIN-1 (mild CED) was observed.

3. The following changes were noted during the 8-week follow-up period after treatment:

- a reduction in the severity of CED signs (leukoplakia, punctuation, acetowhite epithelium, mosaicism, atypical transformation zone);
- improvement in cytological parameters (reduction in leukocytosis, koilocytosis, and number of cells with dyskeratosis);

- significant increase in local immunity parameters compared to baseline (no significant differences between groups in all local immunity parameters at any time point);
- significant decrease in HPV DNA viral load compared to baseline, particularly after the 8-week follow-up, indicating a prolonged antiviral effect of the drugs (no significant differences between groups in HPV DNA viral load at any time point).

4. Both medicinal products were well tolerated, however, the test drug offered a more convenient dosing regimen, unlike the control drug, which was used with the same frequency and required preparation of the solution and tampon application.

Therefore, PROTEFLAZID® suppositories can be recommended as an effective and safe antiviral treatment for gynaecological diseases caused by HPV.

References:

1. Волошина Н. М. (2006) Особливості перебігу папіломавірусної інфекції у вагітних (Клінічна лекція). Репродуктивне здоров'я жінки, 3(28): 82–85.
2. Волошина Н. М. (2007) Цервікальні інтраепітеліальні неоплазії (діагностика, лікування, профілактика). Методичні рекомендації. Запоріжжя, 36 с.
3. Волошина Н. М., Петрова О. Ю., Кузнецова Т. П. та ін. (2007) Моніторинг шийки матки у вагітних. Здоров'я жінки, 2 (30): 63–67.
4. Радіонов В. Г., Шведюк С. В., Семиряд Ю. В. та ін (2002) Застосування Протефлазіду в комплексній терапії папіломавірусної інфекції. Укр. журн. дерматол. венерол. косметол., 4: 86–90.

Reference: Kaminskiy V. V., Shalko M. N., Hrynevych O. Y. (2015). Comparative Efficacy and Safety of PROTEFLAZID® Suppositories and Drops in Patients with Cervical Epithelial Dysplasia Caused by HPV Infection. Ukrainian Medical Journal, 4 (108). VII/VIII. P. 57–59.

3.6. CLINICAL EFFICACY OF TOPICAL USE OF VAGINAL SUPPOSITORIES PROTEFLAZID® IN THE TREATMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA OF MILD AND MODERATE DEGREE CAUSED BY THE HUMAN PAPILLOMA VIRUS

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Abstract

The objective: to study the effectiveness of topical vaginal suppositories PROTEFLAZID® in the form of monotherapy for CIN-I-II, caused by the human papilloma virus (HPV).

Patients and methods. The study involved 50 women with cervical pathology caused by various strains of human papillomavirus (HPV). All the women were examined and treated in Vinnitsia city clinical hospital №1. PAP test based on liquid-based cytology, determining proliferation markers HPV genotyping quantitative estimation, determination of the status of the vagina biocenosis bacterioscopy vaginal discharge performed in the laboratory Synevo.

Results. Own clinical experience vaginal suppositories PROTEFLAZID® as monotherapy for the treatment of cervical intraepithelial neoplasia mild to moderate from HPV infection, the complex evaluation of clinical efficacy based on the study of patients complaints, PAP-test based on liquid-based cytology, proliferation markers p16 and Ki-67, viral load, colposcopy and results of histological examination of altered cervical sites showed a positive therapeutic effect with suppositories, which resulted in 96% of women achieved complete regression of disease, and 4% marked shift in the CIN-II CIN-I.

Conclusion. The positive effect of the drug on the state of the vagina PROTEFLAZID® microbiocenosis, good tolerability, ease of use and lack of side effects give reason for its use in the health care system.

Key words: CIN-I-II, papillomavirus infection, suppositories PROTEFLAZID®.

Introduction

The study of cervical pathology and its possible consequences remains to be the relevant concern in the modern gynecological practice and the important issue of the scientific research and discussions of the scientists and medical practitioners around the world.

A large number of studies have proven that the cause of cervical pathology including cervical dysplasia and cervical cancer is the presence of the human papillomavirus (HPV).

The target cells for HPV are the skin and mucosal epithelial layers. The viruses can exert both productive and transformative effects on the epithelial cells. When the effect is productive, the benign lesions are developed – skin and mucosal papillomas and condylomas. The results of the transformative effect is the cervical epithelial dysplasia – cervical intraepithelial neoplasia (CIN). Its progression leads to the cervical cancer [3]. HPV infection is most common at the age of 16–25 years; however, the infection is transitory in almost 70% of cases. HPV clinical manifestations are most common at more mature age due to the decreased immune defense, which is often accompanied by the processes of pre-tumor and tumor transformation of the affected epithelium. HPV characteristic feature is the ability to cause the proliferation of the skin and mucosal epithelium including the genital tract. There are more than 130 types of papillomaviruses that differ by their DNA structure. Globally, 630 million people are infected with HPV. Five high grade oncogenic HPV genotypes are detected most common: HPV-16 (3.2%), HPV-18 (1.4%), HPV-52 (0.9%), HPV-31 (0.8%) and HPV-58 (0.77%) [1].

However, these numbers reflect only the incidence of HPV clinical manifestations but not the true amount of the infected population, as they do not record the subclinical and latent infections. The papillomavirus infection may progress through several stages:

- 1) primary infection when the virus is localized at the limited anatomical site;
- 2) viral genome persistence in the episomal state accompanied by the production of viral particles during epithelial cell differentiation (re-infection is possible at this stage);
- 3) oncogenic processes as the consequence of interactions of viral oncogenes with the regulatory proteins after viral DNA integration into their genome.

The sexual HPV transmission is the primary route in the women of reproductive age. The HPV is transmitted mainly through the sexual contact, in particular the non-traditional (homosexual, orogenital, anogenital); in addition, HPV can be transmitted transplacentally, intranatally and through non-sexual contact with hands and medical instruments. The most important risk factors of HPV infection are the following:

- early onset of sexual activity;
- having multiple sexual partners;
- existing sexually transmitted infections;
- bad habits;
- somatic diseases associated with the weakened immunity;
- high parity;
- long-term (for more than 5 years) use of hormonal contraceptives;
- hormone-dependent processes in which hyperestrogenemia is observed; long-term use of intrauterine contraception;
- dietary deficiency of vitamins A, C and beta-carotene;
- females whose partners are diagnosed with balanus cancer;
- immunodeficiency conditions including AIDS;
- individual genetic predisposition to gynecological malignant processes [2].

There is a lot of evidence in the literature that the penetration of papillomaviruses occurs at the level of skin and mucosal immature epithelial cells (basal layer). It results in the cellular proliferation but without production of the viral particles, as the proliferating epithelial cells are unable to support the viral life cycle. Complete HPV replication occurs only in

the highly specialized cells of stratified squamous epithelium including the cervical mucous membrane.

Cytology findings of HPV-related lesions that were mentioned in some articles demonstrated that mostly anucleate or orthokeratotic cells are detected in the cellular material.

When the cervical epithelium is infected with papillomavirus, the cells of the stratified squamous epithelium are altered but the immune system does not recognize these infected cells. Therefore, the epithelial cervical cellular structure undergoes the further alterations.

This topic remains relevant and there are many studies and treatment methods of the cervical pathology associated with papillomavirus infection. There is a significant experience of the cervical pathology treatment with the topical use of medicinal products including interferon-containing suppositories. Many treatment regimens and medicinal products with the immunological effect are used. The interferons (IFN) have been proved to be the highly specific cytokines – a group of biologically active proteins and glycoproteids synthesized by the cells in the process of immune reaction in response to the impact of the stimulating agents. The interferons are the important immunity factors, the first line of the anti-inflammatory defense [4, 5]. Interferons play a special role because the induction of their synthesis (primarily by natural killer (NK) cells, monocyte cells and dendritic cells) prevents the formation of specific immune reactions. It is clearly manifested in some viral infections. Like other cytokines, the specific protective effects of interferons are also realized through the signaling cascades.

IFN-alpha and IFN-gamma exert the following effects locally (in infection sites) at the first stage of infection:

- intracellular inhibition of virus reproduction;
- elimination of infected material using NK cells and cytotoxic lymphocytes;
- protection of other uninfected cells from possible damage.

However, the specified effects do not lead to the termination of the infectious process and administration of interferon products is associated not only with a number of side effects but also with the drug tolerance. It results in their further up-titration, for example due to the development of autoantibodies to the exogenous recombinant interferon, which is especially pronounced in the long-term course of diseases that require multiple dosing of interferons at high concentrations. High price of the interferon products is another important factor limiting their use in the broad practice. In this respect, the issue of the availability or absence of the appropriate medicinal products in the market is not as much relevant, as is the issue of the drug resistance developed in viruses. The resistance is acquired only via mutation – when a new genotype is emerged, a new viral strain appears, against which the immune system is defenseless.

The national medicinal product PROTEFLAZID® used as vaginal suppositories and having important characteristic feature distinguishing it from the interferon products is the interesting way to solve the problem associated with virus-induced cervical pathology (CIN-II, CIN-I class neoplasia) in this context. The product exerts the direct antiviral effect on the viral RNA and DNA without causing their mutations; therefore, the viruses cannot become resistant to its active substance. The active substance is composed of flavonoids obtained from the mixture of *Herba Deschampsia caespitosa* L. and *Herba Calamagrostis epigeios* L.,

making the life cycle of these medicinal products unlimited. Flavonoids inhibit DNA polymerase and thymidine kinase (specific viral enzymes) in the virus-infected cells.

Inhibition of these enzymes leads to the terminated replication of viral DNA making viral replication impossible. In addition, PROTEFLAZID® facilitates the improvement of the non-specific immunity increasing the endogenous interferon level and the body's resistance not only to viruses, but also to bacteria. The medicinal product also possesses the antioxidant properties preventing the accumulation of lipid peroxidation products. Therefore, when PROTEFLAZID® suppositories are used topically, all these properties are especially important in the treatment of cervical disorders associated with the papillomavirus etiology.

A virus is a living microorganism parasitizing in the microorganism cells. And if the virus "feeds" on the same things that human cells do, then like a human cell, it perceives the grass components composing the herbal medication PROTEFLAZID® as the phylogenetically common products. The grasses are the natural herbal components that are physiological to the human body rather than chemical structures. Thus, the first component of the general problem (viral mutability with the resistance acquisition) has been solved, in this case due to the herbal antiviral medicinal product that is phylogenetically associated with the human body.

The other important aspect of using the medicinal products in this pathology is its availability in the national pharmacy chains and affordability in comparison with other immunomodulating medications. The ease of medicinal product use is also of great importance.

Study objective

To investigate the efficacy of topical use of PROTEFLAZID® vaginal suppositories as monotherapy in HPV associated CIN-I-II.

Materials and methods

The study involved 50 women with the cervical pathology caused by different HPV strains. All women had examinations and treatment in Vinnytsia City Clinical Maternity Hospital No. 1. The following examinations and tests were performed to evaluate the eligibility and treatment efficacy: review of complaints, Pap test by liquid-based cytology method (BD SurePath technology), detection of p16 and Ki-67 proliferation markers by immunocytochemistry, quantitative genotyping of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 types (analytical sensitivity of the test system to quantify HPV 1–5×10³ copies/ml), colposcopy, histology of altered cervical areas. Those parameters were evaluated pre-treatment and 8 weeks post-treatment.

The women also had the vaginal discharge bacterioscopy test prior to and after each 14-day treatment course (4 tests during the study), pre-treatment – to assess the vaginal discharge microflora by Florocenosis method to rule out the true pathogens (*Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Chlamidia trachomatis*, *Mycoplasma genitalis*) and to assess the vaginal biocenosis (bacterial vaginosis, myco- and ureaplasma infection, vulvovaginal candidiasis, aerobic vaginitis) with detection of 17 pathogens by PCR method. Pap test by liquid-based cytology method, detection of proliferation markers, HPV quantitative genotyping, vaginal biocenosis assessment and vaginal discharge bacterioscopy were performed in Synevo laboratory.

The colposcopy was performed using Scanner MK-300 colposcope (Ukraine) and vascular tests. Colposcopic biopsy was performed at the areas with the marked pathological changes using conchotome.

The material for Pap test and detection of proliferation and HPV markers was taken from the endocervix and exocervix using combi-brush Cervex-Brush Combi. Pap test findings were evaluated using Bethesda system (2014) and correlated with Papanicolaou, CIN and WHO systems. The following was assessed: material quality, presence of specific infectious agents, cellular processes not associated with neoplastic processes (metaplasia, atrophy, etc.), presence of endometrioid cells, reactive changes (inflammation), changes of squamous epithelium: NILM – type I, cytogram is within normal, NILM – type II, inflammatory type of smear, ASC-US – atypical squamous cells of undetermined significance, ASC-H – atypical squamous cells, cannot exclude high-grade lesion, LSIL – low-grade intraepithelial lesion: type III, mild dysplasia, CIN-I/ type III, signs of HPV infection, koilocytosis, HSIL – high-grade intraepithelial lesion: type III, moderate dysplasia, CIN-II/ type III, severe dysplasia, CIN III/ type IV, suspected cancer, carcinoma in situ, CIS – invasive squamous cell carcinoma and changes of glandular epithelium: AGC-US – atypical glandular cells, undetermined significance, AGS favor neoplastic – atypical glandular cells with suspected neoplasia, AIS – adenocarcinoma in situ, adenocarcinoma.

Reference values of proliferation markers involve negative or positive reaction – a result was considered positive only if p16 and Ki-67 were detected simultaneously in one cell indicating the presence of the malignant transformation in the cervical epithelial cells.

The vaginal discharge sample for bacterioscopy was taken using spatula from three points – urethra, posterior vaginal fornix and cervical canal – and then spread on a glass slide with the further Gram stain. The bacterioscopy involved the evaluation of WBC count, amount of squamous epithelial cells, mucus, gram-positive rods – Doderlein lactobacilli, other gram-positive and gram-negative microorganisms and vaginal bacterial contamination.

The material was collected on Day 10 to Day 20 of menstrual cycle, in the absence of bleeding, not earlier than 24 hours after the gynecological examination, colposcopy, sexual intercourse or the use of various vaginal products and not earlier than 3 weeks after the previous cytology.

Inclusion criteria were: presence of cervical intraepithelial neoplasia (CIN), grade I–II associated with different HPV strains, woman's willing and possibility to receive treatment.

Exclusion criteria were: presence of cervical intraepithelial neoplasia (CIN), grade III, vaginal infections and any impairments of the vaginal biocenosis, age up to 23 years (possibility of congenital cervical alterations), pregnancy. The women received topical monotherapy with PROTEFLAZID® vaginal suppositories by the following regimen: 1 suppository twice daily for 14 days monthly for 3 consecutive months. The suppository was to be inserted deep into the vagina. No other vaginal procedures or medical therapy were used. The patients used condom to prevent the re-infection during the whole treatment course and prior to the follow-up examination.

The cryodestruction of altered cervical areas was performed after therapy received if partial regression was observed. Treatment efficacy was evaluated based on the changes of patient complaints, results of the Pap test, proliferation markers, HPV viral load, colposcopy findings and histology findings. Also, medicinal product effect on the vaginal biocenosis, tolerability and side effects were evaluated.

Study results and their discussion

Fifty (50) women were selected from 380 females who consulted the gynecologist for different reasons and had screening cytology by Papanicolaou method; those women were allocated to the study group based on the inclusion criteria.

The study group included 30 (60.0%) patients aged 23–30 years and 20 (40.0%) patients aged 30–35 years. Mean age at the onset of sexual life was 16.8 ± 1.9 years. Majority of women (47–94%) had 2 and more sexual partners. There were 26 (52.0%) nulliparous women; however, every one had reproductive plans for future. All patients refused to have the destructive cervical treatment.

The complaints of women pre- and post-treatment are provided in Table 1.

Pre-treatment complaints included vaginal discomfort – 52%, vaginal discharge – 40%, vaginal itching and burning – 36%, contact bleeding – 20%. However, it should be noted, that every fifth woman (20%) did not have the complaints and cervical alterations were detected only at the cancer screening.

Table 1. Pre- and post-treatment complaints of patients, n = 50

Complaints	Pre-treatment		Post-treatment	
	Abs.	%	Abs.	%
Vaginal discomfort	26	52	2	4
Vaginal discharge	20	40	2	4
Vaginal itching and burning	18	36		
Contact bleeding	10	20		
No complaints	10	20	46	92

After treatment received, 92% of women recorded improvement of subjective sensations and did not have any complaints; 24 (92%) of 26 patients noted decreased discomfort feeling and 18 (90%) of 20 patients with pre-treatment complaints recorded the decreased vaginal discharge.

The species composition of papilloma viruses detected at strain genotyping in cervical smear is presented in Figure 1.

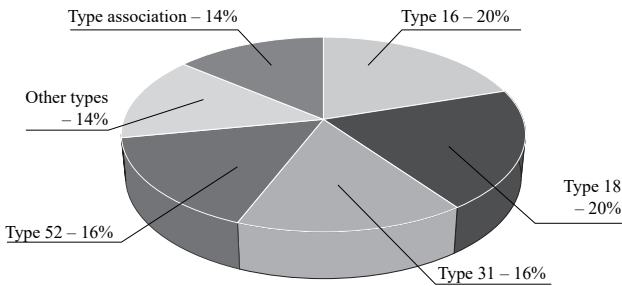


Figure 1. Human papillomavirus genotypes in patients examined, n=50

Figure 1 shows that HPV type 16 and type 18 were mostly detected in women – in 10 (20%) patients each, type 31 and type 52 were less common – in 8 (16%) patients each; other types and associations were detected in 7 (14%) patients. Thus, the majority of women (36 [72%]) were infected with one virus of high cancerogenic risk.

Results of the vaginal discharge bacterioscopy performed pre and post every 14-day treatment course are provided in Figures 2 and 3.

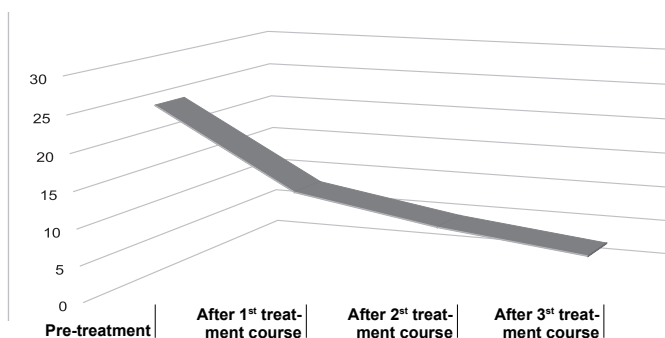


Figure 2. WBC count in vaginal discharge pre-treatment, during treatment and post-treatment

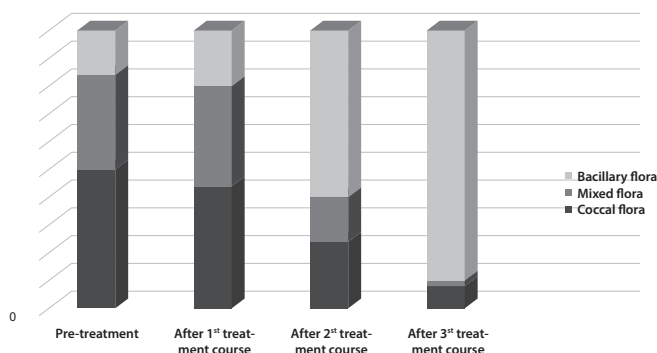


Figure 3. Composition of vaginal microflora pre-treatment, during treatment and post-treatment

As it is seen in Figure 2, WBC count per field of view of the vaginal discharge had the trend towards decrease from 26 ± 2.3 at the treatment initiation to 12 ± 1.8 after the third 14-day course using PROTEFLAZID[®] vaginal suppositories indicating the decreased signs of the vaginal mucosal inflammation secondary to administration of medicinal product.

The test results demonstrated that the coccal flora was predominant in the vaginal discharge of women pre-treatment – in 25 (50%) patients, bacillary flora (*Doderlein bacillus*) was observed only in 8 patients (16%). During the treatment, the number of women with lactobacilli detected was progressively increased and after the third treatment course with PROTEFLAZID[®] suppositories there were 45 (90%) such patients indicating the positive effect of the medicinal product on the vaginal microflora condition and its normalization.

Pap test by liquid-based cytology method was performed pre-treatment and 8 weeks after the last treatment course with medicinal product PROTEFLAZID[®] (suppositories). The obtained results are provided in Table 2.

Table 2. Results of Pap test by liquid-based cytology method pre- and post-treatment, n = 50

Evaluation	Pre-treatment		Post-treatment	
	Abs.	%	Abs.	%
NILM, type I			10	20
NILM, type II			31	62
ASC US	10	20	5	10
LSIL	40	80	4	8

The provided results show that before the treatment, the majority of women – 40 (80%) – had the signs of the mild cervical intraepithelial neoplasia and atypical cells of undetermined significance requiring diagnosis specification were detected in 10 (20%) patients. Eight (8) weeks after 3-cycle treatment course with PROTEFLAZID® suppositories, complete normalization of the cytology findings was recorded in 10 (20%) patients; signs of the cervical intraepithelial neoplasia disappeared in 36 (72%) women. Thus, the positive cytology findings were observed in 92% of patients post-treatment with the medicinal product.

The results of test for p16 and Ki-67 proliferation markers in the cervical materials demonstrated that only p16 was reported pre-treatment in 5 (10%) patients and only Ki67 – in 4 (8%). Women with the positive results for p16 and Ki-67 simultaneously were not enrolled in the study due to the high probability of malignant transformation. Eight (8) weeks after received treatment, no markers were detected in any women.

We also evaluated the viral load pre-treatment and 8 weeks after the third treatment course with PROTEFLAZID® suppositories. The obtained results are provided in Table 3.

Table 3. HPV viral load pre- and post-treatment

Parameter	Viral load (Lg HPV/10 ⁵ cells)
Pre-treatment	5.1 ± 0.35
8 week post-treatment	2.8 ± 0.36
Treatment effect	2.3* ± 0.43

Note: * – $p < 0.05$.

The provided data show the clinically significant HPV load (more than 5 Lg HPV/10⁵ cells) pre-treatment; post-treatment the load in 40 (80%) subjects was significantly ($p < 0.05$) decreased by almost half and was clinically insignificant (less than 3 Lg HPV/10⁵ cells). The virus was not isolated at all in 10 (20%) patients post-treatment indicating its elimination secondary to the treatment and direct antiviral effect of PROTEFLAZID® suppositories.

The changes of the colposcopy findings pre- and post-treatment are provided in Table 4.

Table 4. Colposcopy signs in women pre-treatment and 8 weeks post-treatment, n = 50

Colposcopy signs	Pre-treatment		Post-treatment	
	Abs.	%	Abs.	%
Leukoplakia or keratosis	10	20	2	4
Iodine-negative area	12	24	2	4
Acetowhite epithelium	15	30	4	8
Mosaic pattern	10	20		
Punctuation	8	16		
Columnar epithelium ectopia	10	20	17	34
Normal pattern			25	50

Note: The sum exceeds 100%, as combination of signs was observed.

Analysis of the obtained data shows that 8 weeks after the third course of treatment with PROTEFLAZID® suppositories, the normalization of colposcopy findings was recorded in 25 (50%) women, significant improvement was reported in 17 (34%) women and colposcopy findings remained abnormal only in 8 (16%) patients. The rate of leukoplakia or keratosis detection decreased from 20% to 4%, iodine-negative areas – from 24% to 4%, acetowhite epithelium – from 30% to 8%, mosaic pattern and punctuation were reported in 20% and 16%, respectively pre-treatment and were not detected in any woman post-treatment. Thus, the positive effect of medicinal product on the cervical condition was observed totally in 42 (84%) women from the study group based on colposcopy results.

The histological examination of the altered cervical areas was performed pre-treatment and 8 weeks after the last course of treatment with PROTEFLAZID® suppositories to make the final diagnosis and evaluate the treatment efficacy treatment. The results are provided in Figure 4 and Table 5.

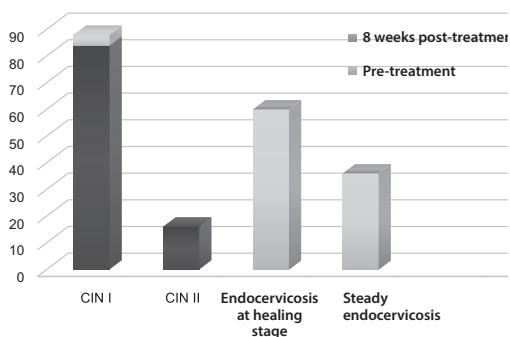


Figure 4. Findings of histology of cervical biopsy material pre- and post-treatment, n = 50

Table 5. Findings of histology of cervical biopsy material pre- and post-treatment, n = 50

Parameter	Pre-treatment		Post-treatment	
	Abs.	%	Abs.	%
CIN I	42	84	2	4
CIN II	8	16	0	0
Endocervicosis at healing stage			30	60
Steady endocervicosis			18	36

Based on the pre-treatment results, the histological findings in 42 women (84%) included mild cervical intraepithelial neoplasia (CIN-I), in 8 (16%) women – moderate (CIN-II). After the last treatment course with PROTEFLAZID® suppositories, the complete CIN regression was detected totally in 48 (96%) patients; morphologically the following was detected: endocervicosis at healing stage – in 30 (60%) patients or steady endocervicosis – in 18 (36%) patients and transition of CIN-I to CIN-II – in 2 (4%) patients, i.e. improvement of the histology findings.

Thus, the comprehensive efficacy evaluation of 3-cycle course of treatment with PROTEFLAZID® suppositories (14 days each) of mild to moderate cervical intraepithelial neoplasia associated with HPV based on the clinical findings, results of Pap test by liquid-based

cytology method, detection of proliferation markers, viral load, colposcopy and histology (allowing the increased accuracy of the obtained data) supported 100% positive effect of the medicinal product with the complete regression observed in 96% of patients and improved cervical condition – in 4%.

The evaluation of tolerability and side effects of PROTEFLAZID® suppositories demonstrated that the medicinal product was well tolerated by all women, the insertion was painless and only 5 (10.0%) of them were concerned that the suppositories were poorly absorbed in the vagina but it did not require any additional activities. All women mentioned the ease of use of the suppositories.

Conclusions

Clinical evaluation of the topical use efficacy of vaginal suppositories PROTEFLAZID® as 3 cycles of monotherapy (14 days each during 3 months) supported the positive effect on the cervical condition based on the results of Pap test by liquid-based cytology method – decreased rate of LSIS detection from 3 80% to 8%, normalization in 82% of patients; colposcopy findings – normal pattern post-treatment was observed in 82% of women; no p16 and Ki-67 proliferation markers detected in any woman; significantly decreased viral load by 1.8 times; morphologically confirmed regression of the cervical intraepithelial neoplasia in 96% and transition of CIN-II to CIN-I in 4% of patients. Also the positive effect of suppositories on the vaginal microbiocenosis was reported and manifested by the decreased WBC count per field of view by 2.16 times and increased rate of lactobacillus detection by 5.6 times. In addition, the women tolerated this treatment method well; it was convenient and painless, received in outpatient settings and practically did not have the side effects.

References:

1. Камінський В. В., Шалько М. М., Воробйова Л. І., Ромащенко О. В., Гриневич О. Й. ПРОТЕФЛАЗІД®: специфічна активність у доклінічних дослідженнях, ефективність та безпека застосування у клінічній практиці при захворюваннях, спричинених вірусом папіломи людини (систематичний огляд) // Здоров'я жінки. – 2015. – №3 (99). – С. 122–132.
2. Годлевська Н. А., Старовер А. В. Досвід використання препарату ПРОТЕФЛАЗІД® в лікуванні патології шийки матки, асоційованою з папіломавірусною інфекцією // Вісник Вінницького державного медичного університету. – 2012. Т. 16, №1. – С. 91–96.
3. Воробйова Л. І. Папіломавірусна інфекція: актуальна проблема сучасної гінекології // Здоров'я жінки. – 2015. – №3 (99). – С. 21–24.
4. Суслікова Л. В., Борис О. М., Суменко В. В., Камінський А. В., Сербенюк А. В. Ефективність застосування та переносимість імуномодуляторів у жінок із папіломавірусною інфекцією, поєднаною з передпухлинними процесами шийки матки та уrogenітальною мікстинфекцією // Здоров'я жінки. – 2009. – №4 (40). – С. 140–146.
5. Карташов С. М., Білодід О. О. Аналіз ефективності дії імуномодуляторів, що використовуються при лікуванні папіломавірусної інфекції // Здоров'я жінки. – 2009. – №4 (40). – С. 40–41

Reference: N. A. Godlevska, A. V. Starover. Clinical efficacy of topical use of vaginal suppositories PROTEFLAZID® in the treatment of cervical intraepithelial neoplasia of mild and moderate degree caused by the human papilloma virus // Woman Health. – 2017. – No.2 (118). – pp. 55–60.

3.7. THE WAYS OF REDUCING DIAGNOSTIC AND THERAPEUTIC AGGRESSION OF THE PATIENTS WITH HPV-INFECTION IN REPRODUCTIVE AGE

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Abstract

The problem of early diagnostics and prevention of cervical cancer is actual in Ukraine. The leading etiologic factor in the genesis of cervical neoplasia and a number of other organs is Human papillomavirus (HPV). The human papillomavirus is sexually transmitted and has high contagiousity. Cancer prevention consists in effective screening, early detection and treatment of pathological changes in the cervix. The aggressive treatment of diseases caused by (HPV) has been replaced by a tactic of a differentiated approach, taking into account to the age of the woman and her reproductive plans.

The objective: was to study the efficacy and tolerability of the combined use of PROTEFLAZID® systemically in drops form and locally in the form of suppositories for 3 months in patients with cervical intraepithelial neoplasia (CIN) of lung and moderate severity (CIN 1 and CIN 2) associated with the human papillomavirus (HPV); determination on the basis of the results of the need for further destructive treatment.

Materials and methods. For the period from July 2016 to September 2017, we examined and treated 86 women with morphologically confirmed intraepithelial neoplasia of the cervix associated with HPV infection.

Results. Based on the performed studies, it was found that 6 months after treatment with PROTEPLASID® systemically and locally for 3 months, regression of CIN was noted in 93% of patients. In all cases, a reduction in viral load of more than 2 Lg of HPV/10³, which is a marker of the effectiveness of antiviral therapy, has been recorded. Six months after treatment in 84% of patients and 9 months in 88%, there was complete elimination of HPV or reduced viral load to clinically insignificant values – less than 3 Lg.

Conclusion. The drug PROTEFLAZID® suppositories and drops contributes to the elimination of human papillomavirus (HPV) and other viral-bacterial infections and reduces the risk of cervical neoplasia.

Key words: cervical cancer, screening, cervical neoplasia, Human papillomavirus, viral-bacterial infections, PROTEFLAZID®.

Introduction

Cervical cancer (CC) is one of the major problems in modern gynecologic oncology. Each year, approximately 5,000 new cases of cervical cancer are registered in our country. Since 1990, there has been a tendency toward increasing incidence and mortality from cervical cancer. From 1997 to 2015, according to data from the Ukrainian Cancer Registry, there was an increase in cervical cancer incidence among young women: ages 21–25 years – from 1.7 to 2.9 cases per 100,000 female population, ages 30–34 years – from 14.6 to 18.5 cases per 100,000 female population. The median age of patients with

cervical cancer *in situ* decreased from 40 to 30 years, which is 10–15 years lower than the corresponding figure for patients with invasive cervical cancer [21, 30].

The issue of early diagnosis and treatment of cervical cancer remains relevant in Ukraine, as almost 25% of CC cases are diagnosed at advanced stages. The mortality rate before cancer becomes evident remains consistently high, serving as an integrated indicator of the quality of CC diagnosis and treatment, and reaches up to 20%. It has been proven that the main etiological factor in the development of cervical neoplasia and neoplasia of some other organs is the human papillomavirus (HPV). HPV is sexually transmitted and has high contagiousness [5, 30, 33].

Over recent decades, significant changes have occurred in the diagnosis, treatment, and prevention of diseases caused by papillomaviruses, particularly cervical cancer [3, 17, 26, 33].

Based on accumulated experience, the aggressive treatment approach to HPV-related diseases has shifted to a treatment tactics of differentiated management, taking into account a woman's age, reproductive plans, and the clinic's capacity to conduct adequate examination and dynamic follow-up.

Papillomaviruses are tropic to epithelial cells of the skin and mucous membranes.

Based on the nucleotide sequence of the L1 gene, which encodes the major capsid protein, over 150 HPV types have been identified and described. These virus types are classified into high-risk and low-risk groups depending on their oncogenic potential [2, 5, 21, 33]. The International Agency for Research on Cancer (IARC) currently recognizes twelve high-risk HPV types associated with human malignancies (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), as well as additional types with limited data regarding their carcinogenicity (types 68 and 73) [4, 17, 33]. Although infection with high-risk oncogenic HPV types is the cause of almost all cases of cervical cancer, these infections do not always lead to cancer. The majority of women infected with high-risk HPV do not develop cancer, because human papillomavirus infection (PVI) is typically transient. On average, the time interval between HPV infection and the appearance of seroconversion is approximately 8–12 months. The majority of HPV infections (70–90%) are asymptomatic and resolve spontaneously within 1–2 years. In some cases, persistent or recurrent HPV infection, caused by high-risk oncogenic types, may progress: pre-cancerous changes may develop, up to invasive carcinoma at the site of the lesion. Chronic HPV infection develops in 5–10% of infected women and is determined by the presence of type-specific HPV DNA in repeated clinical biological samples over 6–18 months. A prolonged course of HPV infection affecting the squamous epithelium of the cervix is histopathologically classified as Cervical Intraepithelial Neoplasia – CIN.

The course of HPV infection largely depends on the state of the immune system.

Given the epitheliotropism of HPV, local immunity factors are of particular importance. Local immunity provides protection of the mucous membranes and skin of the human body against the harmful effects of viruses, bacteria, toxins, allergens, parasites, protozoa, and other harmful agents. Mucous membranes are rich in lymphoid tissue and are densely populated with immunocompetent cells. Epithelial cells of the mucous membranes act not only as a physical barrier against pathogens and infectious agents, but also secrete a wide range of protective factors, such as: lysozyme, lactoferrin, peroxidase, complement components, as well as cytokines and chemokines, which recruit and activate immune cells [6, 8, 11, 15, 16].

The main type of immunoglobulin (Ig) involved in local immunity, especially in maintaining normal microflora, is secretory IgA (sIgA). By binding to microorganisms, it prevents

their attachment to the surface of cells and, together with nonspecific immunity factors, provides protection of mucous membranes from microorganisms and viruses [13, 16, 24]. Lysozyme synthesized by neutrophilic granulocytes is found in vaginal fluid, and in higher concentration in the mucous plug of the cervix. In addition to its antibacterial effect, lysozyme has been shown to block viral entry into the cell and subsequent replication [1, 6, 8, 15]. A reduction in local defense factors is an important element in the pathogenesis of HPV infection, contributing to disease progression.

At present, risk factors for a more aggressive course of HPV infection have been identified. They include:

- the type of HPV and its degree of oncogenicity;
- the immune status of the patient;
- concomitant sexually transmitted infections (STIs), such as herpes simplex virus (HSV), chlamydia, trichomoniasis, and gonorrhea.

Women at increased risk for cervical cancer (CC) include women who have multiple sexual partners, early childbirth (before the age of 18), a large number of births and abortions as well as those who smoke and abuse alcohol [21, 29, 33].

The duration of HPV infection with progression to invasive cancer is typically up to 10 years and more. Therefore, both the patient and physician have sufficient time for diagnosis, treatment, and prevention of neoplasia disease progression [1, 2, 5, 21, 32].

The presence of HPV infection and CIN can be diagnosed using several tests: Pap test (cytological examination); HPV testing (detection of HPV DNA); colposcopy; biopsy followed by histological examination; immunohistochemical examination; biomarker identification.

The informative value of each of these methods depends on a number of subjective factors.

Unfortunately, none of these methods can guarantee 100% detection of the disease.

The informative value of the Pap test largely depends on the patient's preparation for the examination, the quality of cytological material collection by the gynecologist or a trained nurse, proper fixation, the staining method, as well as accurate interpretation of cellular changes by the cytologist. At present, cytological examination remains the primary method of cervical cancer screening in most countries of the world [3, 9, 21, 35].

The use of the modern Bethesda terminology system (The Bethesda System – TBS, 1988, USA), which has been revised and updated in 2001, 2006, and 2014, makes it possible to characterize cytological changes associated with HPV infection [9, 35].

TBS presents a two-tiered system for assessing squamous intraepithelial lesions of low and high grade (LSIL and HSIL), which reflect the biology of changes occurring during HPV infection.

The productive (episomal) stage of HPV infection corresponds to LSIL, and the integrated stage corresponds to HSIL [21, 35].

Currently, in most countries using cytological screening, it is recommended to report cytological findings using the Bethesda System (2014), which includes the following categories for evaluating the squamous epithelium of the cervix:

- NILM – Negative for intraepithelial lesion or malignancy;
- LSIL – Low-grade squamous intraepithelial lesion;
- HSIL – High-grade squamous intraepithelial lesion;

- ASC-US – Atypical squamous cells of undetermined significance;
- ASC-H – Atypical squamous cells, cannot exclude HSIL;
- CIS – Carcinoma in situ;
- SCC – Squamous cell carcinoma.

Issuing a cytological report using the TBS system standardizes the format of cytological conclusions worldwide and allows for the exchange of experience in the management of different types of pathology. Adding the ASC-US/ASC-H categories for squamous epithelium increases the diagnostic sensitivity of cytological screening [21, 27, 35].

For histological examination, the WHO classification and the CIN system (1997) are used.

The informative value of the biopsy depends on the quality and size of the sample, as well as on the number of fragments taken for examination. If the material for examination is collected incorrectly (less than 6 mm in depth or from areas with lower-grade lesions), severe lesions might not be verified. In such cases, the patient is at risk of undergoing destructive influence without subsequent histological control, which may lead to the progression of the malignant process. An important point is the use of targeted (colposcopy-guided) multiple biopsy or loop excision, which increases the likelihood that the most altered epithelial areas will be included in the sample. Based on the results of the histological report, the diagnosis is established and management tactics are determined [21, 28, 32].

HPV testing has high sensitivity, but lower specificity. According to international recommendations (2013, 2014), this method is recommended for cervical cancer screening programs in women over the age of 30, in combination with the Pap test, and also for dynamic monitoring of patients with CIN who have undergone some form of treatment.

In these cases, it is important both to perform HPV testing and determine the virus type, its potential for persistence, and the viral load.

CIN, which are precursors of cervical cancer, occur predominantly in young women of reproductive age. As noted by most researchers, and also based on our own observations, low-grade lesions (LSIL) are more frequently detected in young women, and they regress spontaneously following elimination of the papillomavirus [21, 32].

The frequency of cytological and colposcopic changes caused by transient HPV infection is quite high. The proportion of cervical cancer cases developing before age 21 is minimal and accounts for 0.01% of all cervical cancer cases.

Quite often, based only on colposcopic findings detected on the cervix, young patients undergo unjustified invasive procedures and get destructive treatment prescription.

In the presence of persistent infection against the background of immature epithelium, the majority of patients experience recurrences of the disease and do not receive comprehensive treatment, while the possibility of performing repeated surgical interventions is limited.

Therefore, it is important to correctly interpret colposcopic pictures [21, 28, 29, 33].

The colposcopic classification approved by the XIV World Congress of the International Federation for Cervical Pathology and Colposcopy (IFCPC) in 2011 in Rio de Janeiro summarized new knowledge on the of CIN development and is convenient for practical application.

One of the most important criteria for evaluating colposcopic findings is the visualization of the transformation zone (TZ) and the assessment of the adequacy of the colposcopic examination [28, 33].

According to the ratio of ecto- and endocervical components, three types of transformation zones (TZ) are distinguished:

- TZ type 1 – fully visualized. Located on the exocervix and may be of any size.
- TZ type 2 – not entirely located on the exocervix. Has an endocervical component. Almost fully visualized, may be of any size.
- TZ type 3 – not fully visualized. Has a large endocervical component. The colposcopic examination is unsatisfactory; the junction of the endocervix is not visible.

One of the important issues is the formation of TZ type 3 in young patients after destructive treatment on the exocervix. Pathological processes that develop in the presence of persistent HPV infection in the cervical canal may be inaccessible to visual inspection, not diagnosed in time, and may later lead to the development of endocervical form of cervical cancer.

The colposcopic image of the transformation zone depends on the degree of replacement of columnar epithelium by squamous epithelium and on the nature of metaplasia.

Metaplastic epithelium in the TZ remains thin and glycogen-deficient for a long time.

Therefore, during visual examination and simple colposcopy, it appears reddish.

After treatment with acetic acid, the metaplastic epithelium turns white, and an acetowhite epithelium (AWE) pattern appears. The lower the maturity of the epithelium, the less favorable its appearance under colposcopy. AWE is the most important colposcopic marker of pathological changes in the cervix, including as a marker of HPV infection. This feature is pathognomonic for all grades of CIN and makes it possible to suspect the process at early stages of development.

The intensity of whitening directly correlates with the severity of the pathological process.

An important colposcopic sign is the presence of a zone with coarser changes inside the pathologically altered area (presence of an inner border) [21, 28, 33].

The term iodine-negative zones, unlike the terms “leukoplakia” or “keratosis,” characterizes changes in the stratified squamous epithelium (SSE) that can be detected only after treatment with Lugol’s solution. Iodine-negative zones often represent varying degrees of keratinization of the SSE (often after intense coagulation). However, it is not possible to predict using the colposcopy the condition of the tissue beneath the keratinized layer of the SSE.

Many authors consider it necessary to perform a biopsy from areas of iodine-negative zones [27, 33].

The terms “punctuation” or “dotting” correspond to the older terms “base” or “papillary zone”. Punctuation is a manifestation of atypical epithelial vascularization.

Histological examination of areas with punctuation reveals epithelium with elongated stromal papillae, which contain vascular loops penetrating the epithelium.

The term “mosaic” corresponds to the older term “field”. Histologically, branched stromal overgrowth with vessels inside can be identified. Mosaic represents islands of metaplastic or atypical epithelium, surrounded by vascular lines. To the naked eye, punctuation and mosaic are usually not visible. Under extended colposcopy, they are clearly delineated on the cervix [21, 28, 29].

Normal vessels during the acetic acid test usually disappear for a short time.

Atypical vessels appear as chaotically arranged vessels of irregular shape, which do not respond to acetic acid solution.

The variety of pathological colposcopic patterns, caused by the presence of immature or metaplastic epithelium on the background of HPV infection, causes carcinophobia in the physician, which leads to the prescription of various invasive procedures.

Violation of the integrity of the cervix and cervical canal negatively affects subsequent pregnancy and childbirth. These data must be taken into account when planning the treatment of pathological processes of the cervix, especially in women with reproductive plans [21, 27, 33].

Various treatment methods for patients with CIN have been developed and applied in global practice. Most modern treatment regimens are based on the destruction of affected tissue through electrosurgical excision, laser- or cryodestruction. In recent years, various options of radio wave treatment, argon plasma coagulation, and photodynamic therapy have been introduced.

Unjustified aggressive treatment of the cervix in young patients often leads to the development of iatrogenic pathology, and later to difficulties in realizing reproductive plans.

An analysis of the results of histological examination of cervical tissue fragments obtained after excision showed that this tactic was unjustified in almost 90% of cases.

Before performing invasive diagnostic and therapeutic procedures, it is necessary to identify and eliminate concomitant aggravating factors and to use the most sparing methods in patients of early reproductive age.

The modern management tactics applied for patients with cervical pathology should be based on evidence-based medicine and must be well-grounded. The management tactics for patients with CIN should take into account a number of factors: the age of the patient, the severity of CIN, reproductive plans, somatic condition, and the capabilities of the clinic.

In cases of CIN 1, it is preferable to establish dynamic observation with cytological and colposcopic monitoring. Active management of CIN 1 cases is recommended in the presence of:

- unsatisfactory colposcopy;
- large lesions of the cervix;
- duration of CIN 1 longer than 18 months;
- patient's age over 35 years;
- impossibility of further follow-up.

In cases of CIN 2, excisional therapy must be performed with mandatory histological examination and a detailed description of the resection margins.

In cases of CIN 3, the patient should be referred for consultation with a gynecologic oncologist.

The absence of a unified approach to the diagnosis and treatment of HPV infection and associated diseases makes it possible for practicing physicians to determine management tactics based on personal preferences. This leads to a high rate of errors in the choice of treatment strategy and tactics, and, as a result, to a high frequency of disease recurrence, causing problems with the realization of reproductive function.

In this context, there is an urgent need to search for new methods and improve existing methods of effective conservative and sparing therapy for the treatment of HPV-associated diseases [13, 19, 20].

Considering the etiology of CIN, therapy aimed at eliminating the etiological agents (HPV) is of great significance in the treatment of this pathology. HPV elimination allows for the use of gentle treatment methods, which is particularly important for the patients of reproductive-age who plan pregnancy [18].

One of the prospective directions for the treatment and secondary prevention of HPV infection is the use of natural flavonoids, which have direct antiviral action; systemic and local immunotropic effects; activate apoptosis; and have antiproliferative influence on virus-infected cells [13, 19, 20].

The medicinal product PROTEFLAZID® contains flavonoids obtained from a 1:1 mixture of *Herba Deschampsia caespitosa* L. and *Herba Calamagrostis epigeios* L., and is available in liquid dosage form (extract in the form of drops) and in the form of suppositories. PROTEFLAZID® belongs to the group of direct-acting antiviral drugs – it inhibits the synthesis of DNA and RNA viruses in infected cells by suppressing the activity of virus-specific RNA and DNA polymerases, thymidine kinase, and reverse transcriptase. The antiviral action of the medicinal product PROTEFLAZID® is enhanced by its immunotropic properties. The medicinal product stimulates the synthesis of α - and γ -interferons without the development of refractoriness, which enables its long-term use. PROTEFLAZID® in a reliable and steady manner increases the components and improves the ratio of the main factors of local immunity – sIgA, lysozyme, and C₃ component of complement, which enhances the anti-infective protection of cervical mucus and the mucous membranes of the genital tract overall in the treatment of diseases caused by HPV, herpes viruses, and mixed urogenital infections [9, 13, 17, 19, 23, 38]. Clinical studies have shown that PROTEFLAZID® (suppositories) restores the protective function of the vaginal and cervical mucous membranes by normalizing local immunity factors (sIgA, lysozyme, and C₃ component of complement) [13, 23, 26]. In cases of vaginosis, vaginitis, and inflammatory diseases of the cervix, it promotes the restoration of local immunity and a faster, more effective elimination of the pathogen [17, 20, 22]. The medicinal product also acts as an apoptosis modulator, enhancing the action of apoptosis-inducing factors, promoting the faster elimination of virus-infected cells, and contributing to the primary prevention of chronic diseases on the background of latent viral infections [14, 23, 26].

In our clinic, the medicinal product PROTEFLAZID® in drop form has been widely used for more than 10 years for the treatment of herpetic, papillomavirus, and mixed infections in patients of different ages, their partners, and pregnant women.

Since July 2016, following the release of a new dosage form of PROTEFLAZID® (suppositories), we have actively used a scheme of simultaneous administration of PROTEFLAZID® in drops form orally and PROTEFLAZID® in suppositories intravaginally.

Study objective

To evaluate the effectiveness and tolerability of the combined use of PROTEFLAZID® administered systemically in the form of drops and locally in the form of suppositories over a period of 3 months in patients with cervical intraepithelial neoplasia (CIN) of mild and moderate severity (CIN 1 and CIN 2), associated with HPV; and to determine necessity for further destructive treatment, based on the obtained results.

Study materials and methods

From July 2016 to September 2017, we examined and treated 86 women with morphologically confirmed cervical intraepithelial neoplasia, associated with HPV infection.

Most of them were patients who had undergone initial examinations at their place of residence in women's health clinics or private medical centers. They were referred to us for the purpose of deciding on the necessity of destructive or conservative treatment. Cytological and histological examinations revealed CIN 1 or CIN 2 in all the patients. Using the polymerase chain reaction (PCR) method, the presence of high oncogenic risk HPV DNA was detected in all participants of the study. A mandatory requirement was the patients' willingness to follow the prescribed regimen and duration of PROTEFLAZID® administration, as well as to undergo scheduled check-ups and tests during 6 months after the completion of treatment. For this purpose, before the start of treatment an individual conversation was conducted with each patient explaining the purpose

and method of therapy. All patients were recommended to stop smoking and to use barrier methods of contraception. As a rule, after such a conversation, patients adhere to the physician's recommendations. PROTEFLAZID® (drops) were taken orally by the patients at a dose of 15 drops twice a day before meal for three months continuously. Simultaneously, PROTEFLAZID® (suppositories) were administered deeply into the vagina: 1 suppository twice a day for 15 days after menstruation, over the course of three menstrual cycles. Patients with vaginal biocenosis disorders and the presence of bacterial, fungal, or protozoal infections were included in the study only after completing preparation and a standard course of treatment. After completion of the specified treatment course after 3 and 6 months, all patients underwent control colposcopic, cytological, and PCR examinations. Treatment was considered effective if 6 months after its completion the viral load had decreased to a clinically insignificant level or was undetectable, if colposcopic findings had improved, and if CIN regression was observed as confirmed by cytological and morphological examinations.

Study results and their discussion

The age of the observed patients ranged from 18 to 30 years. The average age was 25 ± 3.2 years. In assessing reproductive history, it was found that 18 women (21%) had deliveries, 12 (14%) had both deliveries and abortions, and 28 (33%) had only abortions. All patients had reproductive plans; therefore, destructive treatment methods were especially undesirable for them. The survey revealed that 58 (67%) of the women smoked (from 2 to 20 cigarettes per day). Barrier methods of contraception were used (irregularly) by only 20% of the observed women. In the gynecological history, the most common conditions were chronic inflammatory diseases of the pelvic organs – in 48 patients (56%); various forms of vaginitis – in 45 (52%); bacterial vaginosis – in 22 (26%); menstrual disorders were identified in 38 (17%) women; and uterine fibroids were found in 7 (8%) women.

Before the start of treatment, the most frequent changes observed during colposcopic examination were as follows: the presence of areas of acetowhite epithelium (AWE) of varying density – in 72 (64%) patients; iodine-negative areas – in 24 (28%); mosaic and punctuation patterns – in 30 (35%); and thin leukoplakia – in 12 (14%) women.

During the control colposcopic examination, all patients showed positive dynamics in the form of a reduction or disappearance of leukoplakia lesions, AWE, mosaic, and punctuation areas. In 57 (67%) of the women, normalization of the colposcopic picture was observed.

Three months after the completion of treatment, colposcopic examination in most patients revealed type I or II transformation zone with areas of columnar epithelium ectopy and a regularly structured vascular pattern that responded well to the acetic acid test.

The dynamics of the Pap test results are presented in Table 1.

Table 1. The dynamics of Pap test results according to the Bethesda classification (2014)

Indicator	Before treatment		3 months after the treatment end	
	Abs. value	%	Abs. value	%
NILM	0	0	68	79
ASC ⁺ US	18	21	0	0
LSIL	54	63	13	14
HSIL (CIN 2)	14	16	5	6

The cytological examination data indicate a regression of the pathological process in the majority of patients. As a result of treatment, during the control Pap test, atypical squamous cells of undetermined significance (ASC-US) were not detected in any of the examined women. In 68 (79%) of the subjects, the PAP test showed a picture of NILM (Negative for intraepithelial lesion or malignancy). Regression of LSIL (CIN 1) and transition of HSIL (CIN 2) to CIN 1 was noted in 59 (87%) out of 68 patients.

During PCR DNA typing in the study group, before treatment, the presence of high-risk oncogenic HPV was found in all patients. The predominant HPV genotypes were as follows: HPV 16 (21%), HPV 18 (16%), HPV 31 (14%), HPV 33 (13%), HPV 45 (5%), HPV 52 (11%), and in one out of five patients (20%), associations of two to four types of HPV were detected.

Viral load was determined before treatment and 6 and 9 months after the end of treatment.

The examination results are presented in Table 2.

Table 2. Viral load dynamics before and after treatment

Lg HPV/10 ⁵	Before treatment		6 months after the treatment end		9 months after the treatment end	
	Abs. value	%	Abs. value	%	Abs. value	%
Not identified	0	0	22	26	52	60
Less than 3	0	0	50	58	24	28
From 3 to 5	38	44	10	12	8	9
More than 5	48	56	4	5	2	2

6 months after the treatment end, all patients, regardless of initial indicators, demonstrated a reduction in viral load of more than 2 lg HPV/10⁵, which is a marker of antiviral therapy effectiveness. After 9 months, HPV elimination was observed in 52 patients (60%), and in another 24 patients (28%), viral load decreased to clinically insignificant levels (less than 3 lg HPV/10⁵). In 2 patients (2%), the viral load had decreased after 9 months, but remained above 5 lg. In both cases, the disease was caused by an association of four HPV types. They were advised to undergo an additional control HPV DNA test after 12 months.

Analysis of viral load dynamics showed that the decrease continued throughout the entire observation period, which indicates a delayed antiviral effect of PROTEFLAZID®.

Therefore, in clinical practice, to assess the effectiveness of therapy, it is advisable to conduct PCR tests no earlier than 6 months after completing the treatment course, with the control test after 12 months.

For patients whose PAP test corresponded to HSIL (5 women) three months after the end of treatment, excisional biopsy of the cervix was performed, followed by histological and immunohistochemical examination.

Six months after the end of treatment, 80 patients (93%) demonstrated improvement in colposcopic and cytological findings. Regression of LSIL (CIN 1) and HSIL (CIN 2) was noted in 85% of patients. This enabled determining reasonable tactics for patients' management, and helped avoid unnecessary pharmacological and invasive interventions.

Treatment tolerance was evaluated based on patients' complaints. During the combined use of PROTEFLAZID® (drops) and PROTEFLAZID® (suppositories) for 3 months, 6 patients (7%) reported mild vaginal itching and burning during the first days of suppository administration, which did not require discontinuation of the medicinal product. These symptoms resolved

spontaneously by day 3 or 4 of treatment. Five patients (6%) at the beginning of treatment complained about the incomplete dissolution of the suppository from the time of insertion to the next dose. After being advised to follow the rule of deep insertion of suppositories, no further complaints regarding incomplete dissolution were reported.

Based on the conducted study, it was established that 6 months after treatment with the use of the medicinal product PROTEFLAZID®, administered both systemically and locally during 3 months, regression of CIN was observed in 93% of patients. In all cases, a reduction in viral load of more than 2 lg HPV/10⁵ was recorded, which is a marker of the effectiveness of antiviral therapy. Six months after treatment end, 84% of patients, and nine months after treatment – 88% of patients, showed complete elimination of HPV or a reduction in viral load to clinically insignificant levels – less than 3 lg. The therapy was well tolerated by the patients. Mild vaginal itching during the first days of suppository administration did not require discontinuation of the administration. As a result of the conducted therapeutic treatment with the medicinal product PROTEFLAZID®, 93% of women did not require destructive methods of therapy, which is important given their reproductive plans.

Conclusions

Based on the presented data, the scheme of simultaneous combined use of PROTEFLAZID® (suppositories) and PROTEFLAZID® (drops) can be recommended as an effective and safe medicinal product for the treatment of patients with cervical intraepithelial neoplasia (CIN) of mild and moderate severity (CIN 1 and CIN 2), associated with HPV.

This treatment scheme allows practicing physicians to reduce unjustified pharmacological and invasive aggressive treatment in the management of patients with HPV-associated diseases.

Successful treatment of CIN is the key to preventing cervical cancer.

References:

1. Amjadi F., Salehi E., Medhdizadeh M. et al.: Role of the innate immunity in female reproductive tract. *Adv. Biomed. Res.* – 2014. – Vol. 3. – P. 1–15.
2. Atkinson W., Wolfe S., Hamborsky J. *Human Papillomavirus // Epidemiology and Prevention of Vaccine Preventable Diseases [The Pink Book: Course Textbook].* – 12th Edition Second Printing, 2012.
3. Arbyn M., Anttila A., Jordan et al. *European guidelines for quality assurance in cervical cancer screening Second Edition*, 2008.
4. Berzolla C. E. Dysplasia and malignancy in endocervical polyps / C. E. Berzolla // *J Womens Health (Larchmt).* – 2007. – Nov. 16 (9). – P. 1317–1321.
5. Bruni L., Diaz M., Castellsague X. *Cervical Human Papillomavirus Prevalence in 5 Continents: Meta-Analysis of 1 Million Women with Normal Cytological Findings // J. Infect. Dis.* – 2010. – Vol. 202 (15 December). – P. 1790.
6. Cole A. M.: Innate host defense of human vaginal and cervical mucosae. *Curr Top Microbiol Immunol*, 2006; 306: 199–230.
7. *Comprehensive Cervical Cancer Control: A guide to essential practice*, 2010, World Health Organization, www://whqlibdoc.who.int/publications/2006/9241547006_eng.pdfua=1.
8. Hein M., Valore E. V., Helmig R. B. et al.: Antimicrobial factors in cervical mucus plug. *Am J Obstet Gynecol*, 2002; 187: 137–44.
9. Herbert A., Bergeron C., Wiener H. et al. *European guidelines for quality assurance in cervical cancer screening: recommendations for cervical cytology terminology. Cytopathology* 2007; 18: 213–219.

10. European guidelines for quality assurance in cervical cancer screening: recommendations for collecting sam Cples for conventional and liquid-based cytology (2007).
11. Fahey J. V., Sentman C. L., Pioli P. A. et al.: Innate and adaptive immunity in female genital tract: cellular responses and interactions. *Immunol Rev*, 2005; 206: 306–335.
12. Jackson S., Mestecky J., Moldoveanu Z. et al.: Appendix I: collection and processing of human mucosal secretions. *Mucosal Immunology*, 3rd edn. Amsterdam, The Netherlands, Elsevier Academic Press, 2005: 1647–1659.
13. Kaminsky V. V., Litus A. I., Grynevych O. Y. et al.: Proteflazid: Efficacy and safety in urogenital herpes virus infection (systematic review). *Science and Education Studies*, 2015; 2 (16): 705–727.
14. Kornatcska A., Revenko O., Grynevych O. et al.: Therapeutic efficacy and tolerability profiles of Proteflazid®, suppository and drops among the patients with herpesvirus infection in the exacerbation phase. *European Journal of Scientific Research*, 2016, 1(9): 506–522.
15. Lee-Huang S., Maiorov V., Huang P. L. et al.: Structural and functional modeling of human lysozyme reveals a unique nona peptide, HL9, with anti-HIV activity. *Biochemistry*, 2005; 44: 4648–4655.
16. Russell M. W., Mestecky J. Humoral immune responses to microbial infections in the genital tract. *Microbes and infection*, 2002; 4: 667–677.
17. Zaporozhan V. N., Marichereda V. G., Dimcheva L. I. Застосування флавонових глікозидів у терапії генітальної папіломавірусної інфекції у жінок // Application of flavonivuh glycosides papilomavirushnoy therapy of genital infection in women. *Journal of Health Sciences*. 2014; 4 (12): 93–104. ISSN 1429–9623 / 2300–665X.
18. Ventskiy B. Proteflazid® in the treatment and prevention of human herpesvirus infection in pregnant women: a meta-analysis of clinical trials results / B. Ventskiy, O. Grynevych, MD, V. Mikhailov, M. Lesnikova, L. Yaschenko, T. Broun // *American Journal of Science and Technologies*. – 2016. – №1 (21). – January–June. – P. 983–1002.
19. Басв А. І., Кузієва Г. Д., Сілаєв Д. В. та ін: Обґрунтування застосування Протефлазиду® у комплексній терапії мікст-ПСПШ (Повідомлення 3). *Питання дерматології та венерології*, 2011; 3–4 (47–48): 31–33.
20. Бенюк В. О., Гриневич О. Й., Усевич І. А. та ін.: Терапевтична ефективність супозиторіїв Протефлазид® при змішаній уrogenітальній вірусно-бактеріальній інфекції. *Здоров'я жінки*, 2016, 2 (106): 162–166.
21. Волошина Н. М., Волошин Н. А. Скринінг та профілактика раку шийки матки. – Запоріжжя: Друкований світ, 2010. – С. 155.
22. Вовк І. Б., Ревенько О. О., Данилюк О. І. Використання флавоноїдів у комплексному лікуванні жінок із запальними захворюваннями геніталій вірусно-бактеріальної етіології. *Здоров'я жінки*, 2002; 4 (12): 43–45.
23. Войтенко Г. М., Коханов І. В. Фармакоекономічне обґрунтування застосування препарату Протефлазид® у терапії папіломавірусної інфекції // «Рецепт». – 2016. – Т. 19, №3.
24. Годлевська Н. А. Ефективність системного та місцевого застосування препарату Протефлазид® у лікуванні патології шийки матки, спричиненої папіломавірусною інфекцією / Н. А. Годлевська, А. В. Старовір // *Здоров'я жінки*. – 2012. – №3 (69). – С. 80–83.
25. Годлевська Н. А. Клінічна ефективність місцевого застосування вагінальних супозиторіїв Протефлазид® у лікуванні цервікальної інтраепітеліальної неоплазії легкого та помірного ступенів, зумовленої вірусом папіломи людини / Н. А. Годлевська, А. В. Старовір // *Здоров'я жінки* – 2017. – №2 (118). – С. 55–60.

26. Камінський В. В., Шалько М. Н., Воробйова Л. І. та ін: Протефлазид®: специфічна активність у доклінічних дослідженнях, ефективність та безпека застосування в клінічній практиці при захворюваннях, спричинених вірусом папіломи людини. Здоров'я жінки, 2015 року; 3(99): 128–138.
27. Лигирда Н. Ф., Свиницький В. С. До питання організації скринінгу раку шийки матки в Україні //Медичні аспекти здоров'я жінок – 2016.–6 (103).– С. 69–73.
28. Лигирда Н. Ф. Практична кольпоскопія. – К., 2017. – 198 с.
29. Маршета Ж., Декамп Ф. Кольпоскопія. Метод та діагностика / Пер. з франц., за загальною ред. Прилепський В. М. – М.: МЕДпресінформ, 2009. – 200 с.
30. Патологія шийки та тіла матки: керівництво для лікарів акушерів-гінекологів, онкологів, цитологів, гістологів, викладачів та студентів мед. ін-тів/під ред. Є. В. Коханевич. – Ніжин: Гідромакс, 2009. – 350 с.
31. Рак в Україні, 2014–2015. Захворюваність, смертність, показники діяльності онкологічної служби //Бюлетень національного канцер-реєстру України.– 2016. – №17. – С. 48–50.
32. Роговська С. І. Папіломавірусна інфекція у жінок та патологія шийки матки. – М.: ГЕОТАРМедіа, 2005 – 141 с.
33. Роговська С. І. Практична кольпоскопія. – 3 видавництво, випр. та доповн. – М.: ГЕОТАРМедіа, 2013. – 240 с.
34. Ромащенко О. В., Руденко А. В. Протефлазид® в комплексній терапії запальних захворювань геніталій у жінок. Педіатрія, акушерство та гінекологія, 2002; 3: 89–94.
35. Рикова О., Богомаз Т. Термінологічна система ТBS 2014 року. Що нового? З турботою про жінку, 2017; 1 (76): 5–6.
36. Таран О. А., Булашенко О. В. Сучасні аспекти комбінованої терапії цервікальних неоплазій, асоційованих з папіломавірусною інфекцією. Здоров'я жінки, 2014; 2(88): 38–42.
37. Тітмуш Е., Адамс К. Шийка матки. Цитологічний атлас/Переклад з англ., за ред. Н. І. Кондрікова. – М.: Практична медицина, 2009. – С. 255.
38. Kaminsky V. V., Chernyshov V. P., Grynevych O. Y. et al.: Proteflazid® and local immunity in diseases caused by human papillomavirus and mixed urogenital infections. Pol Med J, 2017; XLII(249): 110-115

Reference: Voloshyna N. M., Zvantseva E. D. The Ways of Reducing Diagnostic and Therapeutic Aggression of the Patients with HPV Infection in Reproductive Age // Women's Health. – 2017. – No.9 (125). – pp. 51–58.

3.8. RESULTS OF THE CLINICAL AND MOLECULAR-HISTOLOGICAL ASSESSMENT OF THE EFFECTIVENESS OF THE DRUG PROTEFLAZID® IN THE TREATMENT OF PAPILOMAVIRUS INFECTION IN WOMEN WITH CERVICAL INTRAEPITHELIAL NEOPLASIA

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Abstract

The article presents the results of a clinical and molecular-histological assessment of the effectiveness of the simultaneous use of oral and vaginal forms of the drug PROTEFLAZID® in the treatment of cervical HPV infection in women, which contributed to the eradication of HPV in 76.7–86.7% of cases, and in combined HPV- infections with cervical intraepithelial neoplasia of a mild degree (CIN I) – in 76.7% of patients until its persistent regression, which was due not only to the antiviral effect of PROTEFLAZID®, but also to its effect, as shown by the results of an immunohistochemical study, on the reduction of the expression of nuclear proteins p16 and Ki-67, which are reverse regulators of the cell cycle and significantly increase when cervical epithelial cells are affected by HPV.

It was concluded that the drug PROTEFLAZID® is currently one of the most effective drugs for the treatment of HPV infection, which can be recommended for the eradication of papillomaviruses in women with both low-risk cervical intraepithelial neoplasia (CIN I) and positive HPV-tests with a normal Pap test.

Key words: papillomavirus infection, cervical intraepithelial neoplasia, morphological and immunohistochemical studies, proteins p16 and Ki-67, PROTEFLAZID®.

Introduction

Cervical intraepithelial neoplasia (CIN), or cervical dysplasia, is a precursor to cervical cancer (CRC), which ranks second among malignant neoplasms in women. The tendency to increase mortality from CRC, the complexity and expense of treatment, and unsatisfactory immediate and long-term treatment results determine the relevance of timely diagnosis and treatment of precancerous diseases of the cervix [6, 12, 14, 16, 24, 29]. Determining the factors of malignant neoplasms, early diagnosis and timely treatment of precancerous conditions became key points of the decision of the National Security and Defense Council of Ukraine dated December 22, 2023 “On prevention, early diagnosis and treatment of malignant neoplasms”, which was put into effect by Decree of the President of Ukraine No. 842/2023.

Nobel Prize winner Harald zur Hausen convincingly proved almost 40 years ago that the leading role in the etiology of CIN and cervical cancer belonged to the human papillomavirus

(HPV), which was detected in almost all cases (99%) of squamous cell malignancy of the female genital tract. The high contagiousness of HPV and sexual transmission with a 65–70% probability of partner infection gave every reason to classify cervical cancer as a sexually transmitted disease [17, 25, 27, 28].

Cervical cancer is usually associated with HPVs of the so-called “high carcinogenic risk” (HPV HCR), which include 16, 18, 31, 33, 35, 39, 45, 50, 51, 53, 55, 56, 58, 59, 68 of the serotypes, but on the European continent, papillomaviruses of types 16 and 18 are more common in women with CIN and cervical cancer [12, 25]. It is known that the virus is able to infect only proliferating cells, more often in the zone of transition of the cylindrical epithelium of the cervical canal into the stratified squamous epithelium of the exocervix, where basal cells are close to the surface [28]. With prolonged persistence of HPV in the cervical epithelium, its integration into the genome occurs, and with a certain set of risk factors, the process of oncotransformation of cells can be completed in 5–10 years, but sometimes much faster [12, 18].

The frequency of HPV-infected women in the population varies significantly depending on age, social status, and region, and, according to various authors, ranges from 40 to 87.9% [25, 26]. There is evidence that 15–28% of women with a negative cytological Papanicolaou test (PAP test) but with the presence of HPV DNA may develop CIN II-III within two years, while in HPV-intact women – only in 1–3% of cases, which allows us to classify all clinically healthy women infected with HPV as at risk for cancer [27, 29].

WHO's recognition of the papillomavirus nature of cervical cancer has become the basis, on the one hand, for the development and use of specific HPV vaccines in adolescents and healthy women for the prevention of cervical cancer, and on the other hand, for recommendations on HPV testing in women along with the PAP test (cotesting) to increase the sensitivity of diagnosis and prognosis of CIN, as well as resolving the issue of choosing a treatment for HPV infection [11, 12, 18, 20]. And while the results of the PAP test and histological examination of cervical biopsies have now established a clear position on the need for surgical excision of affected cervix areas in the presence of high-risk cervical intraepithelial lesions (HRCIN according to the Bethesda classification) and which were considered true precancerous conditions [12, 20, 21], constructive approaches to the management of women with initial manifestations of dysplastic cervical lesions on the background of HPV infection, which include koilocytosis and low-risk squamous intraepithelial lesions (LRCIN), have not yet been defined. Discussions on this issue are based on clinical observations that prove that HPV-induced CIN I in women often regresses on its own, therefore, passive dynamic observation tactics are most often used for this group of patients, and if there are no cytological signs of regression, or progression of dysplastic lesions of the cervical epithelium occurs within the next 12–18 months, then excisional treatment methods are recommended for such women [12, 20, 21]. However, it is known from literature sources that in such women, on the background of HPV infection, LRCIN persists in 40–50% of cases, disease progression occurs in 11–25% of cases, and cervical cancer occurs in 1% [25, 26]. Therefore, the question arises whether the doctor has the right to risk the health of women with HPV infection, passively waiting for its possible consequences? It should also be noted that passive follow-up of HPV-infected women contributes to the spread of infection, since the probability of infection of a sexual partner during contact is almost 100% and only 10 copies of HPV DNA are sufficient for this. Condom use reduces

the risk, but does not protect against HPV infection, because the virus can be transmitted from skin to skin and penetration during sexual intercourse is not required for infection [25, 26, 29].

The logic of approaches to the management of any infection is either prevention of this infection, which is provided by vaccination against known pathogens, or, if the infection is already present, eradication of dangerous pathogens with medications. Therefore, it is necessary to once again pay attention to the pathogenesis of precancerous conditions and cervical cancer caused by HPV, which consists of several sequential stages from the moment of infection of a healthy woman, the emergence of low-risk dysplastic lesions of the cervical epithelium (koilocytosis, LRCIN) against the background of HPV infection, with subsequent progression to high-risk HPV lesions of the cervical epithelium (HRCIN) and their subsequent tumor transformation with clinical signs of invasive cervical cancer [21].

In our opinion, the key block of the pathogenesis of carcinogenic HPV lesions of the cervix, which is represented by the primary episode of HPV infection and its morphological consequences in the form of LRCIN, today requires special attention, both from the standpoint of the possibility of preventing the progression of dysplastic lesions of the cervix due to HPV eradication with antiviral medications, and from the standpoint of reducing subsequent material costs for the diagnosis and surgical treatment of LRCIN with sometimes undesirable consequences for reproductive function [2].

In the treatment of HPV infection, two main directions can be distinguished, one of which is associated with the use of drugs that increase local cellular resistance and immunity (interferons and interferon synthesis inducers) [3, 13, 23], and the second – with the use of antiviral drugs that can block virus replication and cause their death [7, 8, 15, 19].

One of the most promising areas in the treatment of HPV is the use of antiviral agents, which include flavonoid extract from a mixture of herbs *Herba Deschampsia caespitosa* L. and *Herba Calamagrostis epigeios* L. as part of the domestic drug PROTEFLAZID® [12, 20], which belongs to the pharmacological group of direct-acting antiviral drugs (ATC code J05A X) for etiopathogenetic therapy of diseases caused by RNA and DNA viruses. This drug is available in two dosage forms: PROTEFLAZID® (drops) for oral use and PROTEFLAZID® (suppositories) for vaginal use, which allows for systemic and local treatment of HPV infection.

According to the recommendations of the National Consensus on the Management of Patients with CIN Caused by HPV Infection [12], systemic and local use of the drug PROTEFLAZID® may be useful for the treatment of HPV infection, successful therapy of CIN I (LSIL) and even CIN II (NSIL) in young women without surgical intervention, as well as before and after surgical treatment of CIN to reduce the frequency of recurrences.

Currently, information is available on the results of more than 20 independent preclinical and clinical observations during the treatment of HPV-associated diseases [5, 7, 8, 15, 19], which indicate that flavonoids in the drug PROTEFLAZID® can:

- inhibit virus-specific enzymes, DNA and RNA polymerase, thymidine kinase, reverse transcriptase, leading to the cessation of viral DNA replication and its reproduction;
- prevent the integration of viral DNA into the genome of the epithelial cell, which significantly reduces the risk of degeneration of virus-infected cells into tumor cells, and also promotes the regression of CIN;

- reduce the expression of oncogenic peptides and stimulate the synthesis of antitumor proteins p53 and pRb;
- increase the synthesis of alpha and gamma interferons, restore indicators of local immunity – lactoferrin, secretory IgA, lysozyme, and C₃-component of complement, and, in this way, contribute to the restoration of the barrier function of the cervical epithelium;
- increase the synthesis of the key apoptosis enzyme – caspase 9, which contributes to the elimination of infected cells.

Objective of the study

Clinical and molecular-morphological evaluation of the efficacy of the drug PROTEFLAZID® when used in the treatment of HPV infection in women with cytological koilocytic reaction, as well as in combination with mild CIN of the cervical epithelium.

Materials and research methods

The study involved 90 women aged 20 to 35 years, who were divided into 3 groups based on the results of the Pap test and HPV test. Group I (control) included 30 women with a negative HPV test and no CIN, Group II included 30 women with a positive HPV test without CIN, and Group III included 30 women with CIN I on the background of HPV infection.

When examining patients and determining the severity of CIN, the recommendations of a number of regulatory documents were taken into account [20, 21, 22].

HPV testing was performed in the Synevo laboratory using the polymerase chain reaction (PCR) method to detect high-risk HPV DNA types 16, 31, 33, 35, 18, 39, 45, 59, 52, 56, 58, 66 in material from the ecto- and endocervix. Repeated testing for the presence of HPV DNA (to monitor the effectiveness of the treatment) was performed no earlier than 5-6 weeks after the course of treatment. Histomorphological and immunohistochemical (IHC) studies of cervical biopsies were performed at the Department of Pathomorphology of the Diagnostic Center of the Dnipro State Medical University using standard methods [1] in women of groups II and III to verify the diagnosis, in women of group I (control) – for differential diagnosis in case of an indeterminate PAP test and its discrepancies with the results of colposcopy. Histological and immunohistochemical studies of cervical biopsies in women of groups II and III were repeated after the end of a 3-month course of treatment. Cytological signs of HPV infection included the presence of koilocytic atypia in the cells of the superficial and intermediate layers of the stratified squamous epithelium of the cervix.

IHC study of intracellular biomarkers using monoclonal antibodies makes it possible to objectively assess the nature of the course of HPV infection and CIN, as well as the effectiveness of their treatment. One of such biomarkers is the p16 protein, the role of which is to negatively regulate cell proliferation through participation in the Cdk-RbE2F signaling pathway, which controls the transition of cells from the G1 phase to the S-phase of the cell cycle. The diagnostic significance of the p16 marker is that it is not detected in normal epithelial cells, but is expressed only by dysplastic cells that replicate HPV. It is also believed that p16 is a marker of the completion of oncotransformation processes; therefore, it is attributed to early signs of precancerous changes in the epithelium of the exocervix and cervical canal [4, 9].

The second biomarker is the nuclear protein Ki-67, the IGH study of which allows for fairly accurate determination of cells that have exited the G0 phase and entered the mitosis pathway, which indicates the important role of Ki-67 in the diagnosis, prognosis, and determination of the effect of treatment of HPV-associated cervical pathology [9, 10, 23].

In the process of preparation for the IGH study, paraffin sections of samples with a thickness of 4–6 μm , obtained on a Microtom HM-340, were applied to adhesive SuperFrost Plus slides and deparaffinized with subsequent heat induction (heat induction of epitope retrieval) in citrate buffer with $\text{pH}=6.0$ in an autoclave (8 minutes at a temperature of $+121^{\circ}\text{C}$).

As primary antibodies, monoclonal antibodies to the proliferation marker Ki-67 (clone MIB-1, DAKO; clone SP6, LabVision) and p16 (kit No. K5334, DakoCytomation) were used. The antibody titer was selected individually for each marker using Antibody diluent (DakoCytomation) as a solvent. The next stage of immunohistochemical study was performed using the latest generation imaging system UltraVision LP (LabVision) and LSAB2, EnVision (DAKO).

Secondary antibodies containing a large number of horseradish peroxidase molecules were applied to the sections and incubated in humid chambers for 30 minutes with washing in TRIS buffer solution between each stage for 10 minutes. Identification of the reaction was carried out by applying a chromogen (DAB, LabVision) under microscope control for 20 seconds to 3 minutes, the manifestation of which was the staining of specific structures depending on the marker (intranuclear or cytoplasmic reaction). To differentiate tissue structures, the sections were additionally stained with Mayer's hematoxylin for 1–3 minutes. Their subsequent dehydration and inclusion in the balsam were carried out according to standards.

A positive IGH reaction with antibodies to Ki-67 was manifested by brown staining of cell nuclei of varying intensity. The most common form of a positive IGH reaction to p16 was brown simultaneous staining of nuclei and cytoplasm (mixed positive reaction).

Cells positive for marker expression were examined in at least 4–6 randomly selected fields of view of a microscope of histological sections using $\times 100$, $\times 200$, $\times 400$, and $\times 1000$ objectives (when necessary). After counting 300 histologically identified objects (nuclei or cells), expression indicators were calculated based on the results of all studied areas. As positive, the reaction in normal cells of the epithelial layer, as well as dysplastically changed ones, was considered.

Proliferation indices (PI) based on Ki-67 protein expression were calculated as the percentage of cells with an intranuclear reaction, regardless of staining intensity, to the total number of cells, on average, based on the results of all studied areas.

The following scale was used to assess p16 protein expression: negative reaction – up to 5% of positively stained cells; focal – up to 80% of cells with positive staining; diffuse – more than 80% of cells with an immunohistochemical label.

Statistical processing of the results was performed using Microsoft Excel (Office Home Business 2KB4Y-6H9DB-BM47K-749PV-PG3KT) and the STATISTICA 6.1 software (StatSoft Inc., serial number AGAR909E415822FA). Analysis of the obtained data with assessment of the statistical probability of differences was performed using parametric and non-parametric statistical methods. The critical value of the statistical significance level when testing all null hypotheses was taken to be equal to 0.05 (5%).

For the treatment of HPV infection in women of groups II and III, with informed consent, we used the drug PROTEFLAZID® according to the following regimen: 15 drops 2 times a day continuously for 3 months and simultaneously 1 suppository intravaginally 2 times a day for 14 days each month for 3 months.

According to several studies [8, 15], this scheme is the most optimal, since when used intravaginally, the drug PROTEFLAZID® (suppositories) affects HPVs located in the surface layers

of the cervical epithelium, and when used orally, PROTEFLAZID® (drops) enters the basal cells through the bloodstream.

Results and discussion

In HPV-infected women of groups II and III, no differences in the frequency of detection of different HPV types were found ($p>0.05$). The predominant type of virus in the examined patients was HPV types 16 and 18, which were detected, respectively, in 35 (58.3%) and 8 (13.3%) of 60 patients (groups II and III together). Other HPV types were less common: type 45 – in 5 (8.3%) of the examined patients, type 31 – in 4 (6.7%), type 33 – in 3 (5.0%), type 58 – in 2 (3.3%). In isolated cases, HPV types 39, 56, and 51 were detected. Other known HPV types (35, 52, 59, 68, 73, and 82) were not found in the study.

In 7 (23.3%) patients of group III, two or more types of HPV were detected during the examination. The most common combination was the combination of types 16 and 18, in 5 (16.7%). The quantitative determination of HPV in group I was 5.1 ± 0.3 Lg HPV/105 cells, in group II, 6.16 ± 0.4 Lg HPV/105 cells.

The assessment of the effect of the drug PROTEFLAZID® on HPV eradication was carried out according to the results of the HPV test after the end of treatment in women of groups II and III (Table 1). As shown in Table 1, treatment of HPV infection with the drug PROTEFLAZID® led to eradication of HPV in 26 (86.7%) of 30 women in Group II and in 23 (76.7%) of 30 women in Group III, which indicates a fairly high percentage of cases of complete removal of the pathogen after a 3-month course of treatment.

Negative consequences of HPV infection treatment in 4 (13.3%) of group II and 7 (23.3%) of group III women were recorded mainly in women infected with HPV type 16, although as a result of simultaneous oral and intravaginal use of the drug PROTEFLAZID®, the viral load in both groups decreased from 5.1 ± 0.3 Lg HPV/105 cells to 4.2 ± 0.1 Lg HPV/105 in group II and from 6.16 ± 0.4 Lg HPV/105 cells to 5.2 ± 0.3 Lg HPV/105 cells in group III women.

Table 1. Results of HPV testing and morphological examination of cervical biopsies in women with HPV infection after a course of combined treatment with the drug PROTEFLAZID® (n,%)

Groups	n	HPV test after the treatment course		Results of morphological examination of cervix biopates	
		Negative	Positive	CIN regression	CIN I
II (without CIN)	30	26 (86,7%)	4 (13,3%)	-	-
II (CIN I)	30	23 (76,7%)	7 (23,3%)	23 (76,7%)	7 (23,3%)

According to the results of cytological and morphological examination of the cervical material, carried out after a course of combined treatment with the drug PROTEFLAZID® according to the proposed scheme, there was a complete recovery in 23 (76.7%) women with CIN I (group III) with complete restoration of the stratified epithelium of the cervix with the elimination of signs of dysplasia – the absence of koilocytosis, acanthosis, giant nuclei, and epitheliocytes with multiple nuclei. It should be noted that the observation of this group of patients over the next 24 months showed the absence of recurrence of the disease in the treated patients. A sample of a positive result of treatment of CIN I against the background of HPV with the drug PROTEFLAZID® according to the data of examination of the cervix during colposcopy is presented in Figure 1.

To determine the molecular mechanisms of the effect of the drug PROTEFLAZID® on the course of CIN on the background of HPV in all women of the studied groups, an IGH study was performed in cervical epithelium biopsies using monoclonal antibodies to the p16 and Ki-67 oncoproteins.

The p16 oncoprotein is a naturally nuclear protein, and in women of the control group (group I), in the cervical epithelium, it was determined only as one type of IGH staining, namely intranuclear, which we attributed to a negative status of the expression of this marker.

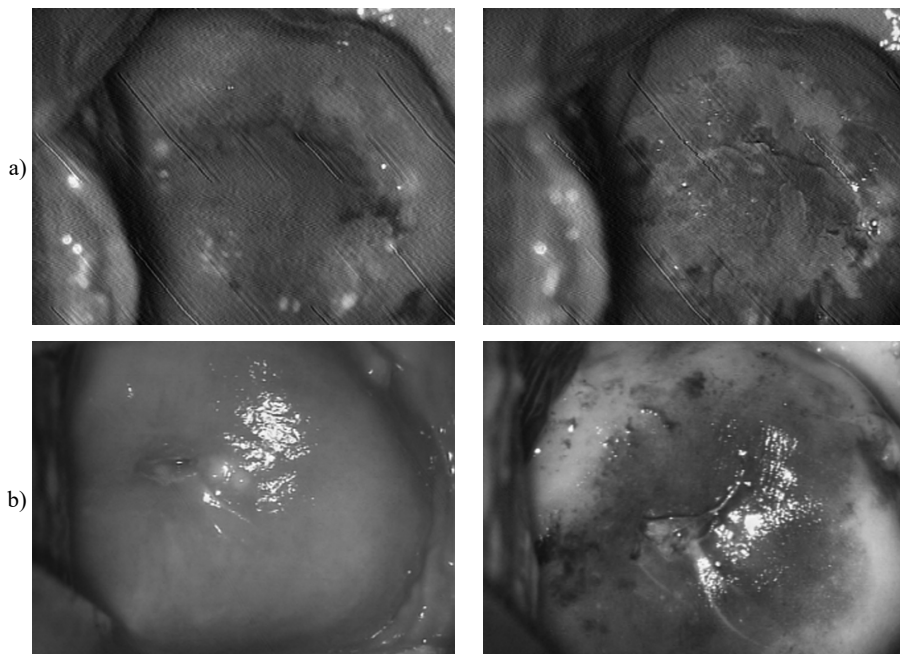


Fig. 1. Native image of the cervix (left) and after testing with Lugol's solution (right): a) before treatment; b) after treatment with the drug PROTEFLAZID®

In women with HPV without CIN (group II), along with a predominantly negative status for p16 expression, in 7 (23.3%) cervical epithelium samples, there were also cases of focal positive IGH reaction of weak intensity simultaneously in the nuclei and cytoplasm (mixed positive reaction), mainly in cells of the basal and parabasal layers of the cervical epithelium. After a course of therapy with the drug PROTEFLAZID® according to the prescribed regimen, only isolated cases with a weak IGH reaction in less than 5% of basal layer cells were found to be positive for p16 in cervical preparations, as in healthy women (group I).

In women with CIN I (group III), out of 30 examined cervical epithelium samples, 10 (33.3%) showed a diffuse positive IGH reaction to the p16 antigen (Fig. 2, a); in 14 (46.7%) samples, IGH staining was focal, and the remaining 6 (20.0%) samples were negative for the p16 marker. After a course of treatment with the drug PROTEFLAZID®, the number of samples with a positive diffuse IGH reaction to p16 significantly decreased. In particular, out of 10 samples with diffuse IGH staining

with the p16 antigen, this nature of its expression was preserved only in 4 (40.0%), i.e., the number of such cases decreased by 2.5 times. In the other 4 cervical biopsy samples, IGH staining for the p16 protein acquired a focal nature with a cytoplasmic reaction of weak intensity, mainly in the cells of the basal and parabasal layers of the cervical epithelium (Fig. 2, b), and 2 samples became IGH-negative.

Of the 14 preparations with focal IGH staining in the reaction with the p16 protein, the majority of control samples of the cervical epithelium with CIN I after treatment became negative according to the results of the IGH reaction (8 cases – 57.1%), i.e. in this category of assessment of p16 expression, the number of cases with a focal reaction decreased by 1.8 times. In general, taking into account all positive cervical epithelial samples in the IHC reaction with p16, both focal and diffuse, after treatment of HPV infection in women with CIN I, 14 (58.3%) cervical epithelial samples became negative for the expression of this marker. Thus, combined treatment of HPV infection in women with CIN I with the drug PROTEFLAZID® helps to reduce the number of cases of positive cervical cells with p16 protein expression by almost half.

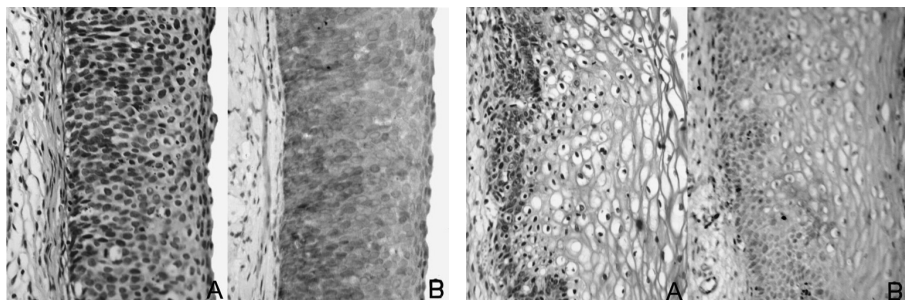


Fig. 2. Character of IGH reaction with protein p16 in the epithelium of cervical samples with CIN I: a) before treatment – intranuclear diffuse reaction; b) after treatment with PROTEFLAZID® – intranuclear focal reaction (IGH, DAKO EnVision imaging system. Magnification x400)

In samples of the cervical epithelium of healthy women (group I), the number of IGH-positive cells expressing the Ki-67 protein did not exceed 3%.

In the presence of HPV infection but without CIN (group II), the number of positive cells expressing Ki-67 ranged from 5 to 12%, and in combination with CIN I (group III) – from 5% to 20%, they were located mainly in the parabasal layer, individual cells with an intranuclear reaction were also found in the basal layer. With cytological signs of HPV infection (koilocytosis), we observed cells with a high level of Ki-67 expression in almost all layers of the cervical epithelium (Fig. 3, a).

The average value of the proliferation index determined by the expression of the Ki-67 protein in cervical biopsy samples in women with CIN I (group III), which, according to the calculation of positive cells, was $19.7 \pm 0.7\%$, significantly exceeded the corresponding value (3.2 ± 0.4 ; $p < 0.05$) in normal epithelium (group I). During treatment with the drug PROTEFLAZID® in these women, there was a significant decrease in this indicator to $9.2 \pm 0.5\%$ ($p < 0.05$), i.e., almost 2 times, and the expression of the Ki-67 protein was determined only as an intranuclear focal IGH reaction in the parabasal layer of the cervical epithelium (Fig. 3, b). In women of group II with HPV infection and the presence of only cytological signs of koilocytosis, after treatment, the average value of the proliferation index as determined by the IGH marker Ki-67 decreased from $6.8 \pm 0.2\%$ to the values in the control group I of women (3.2 ± 0.4 ; $p < 0.05$).

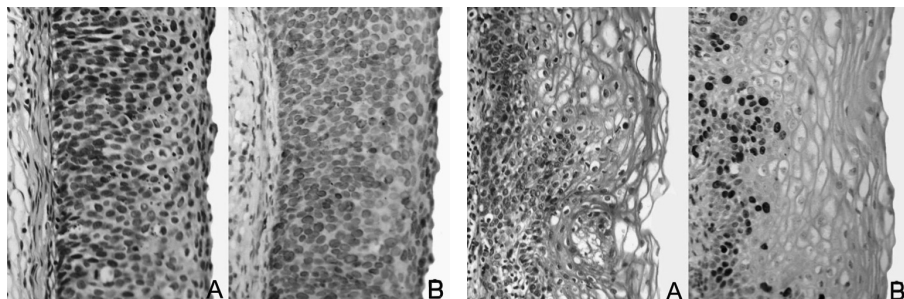


Fig. 3. Character of IGH reaction with Ki-67 protein in the epithelium of cervical samples with CIN I:

- a) before treatment – intranuclear reaction in all layers of the integumentary epithelium;
- b) after treatment with PROTEFLAZID® – intranuclear focal reaction in the parabasal layer (IGH, DAKO EnVision imaging system. Magnification x400)

In this way, the IGH determination of intracellular markers p16 and Ki-67 proved that under the influence of the drug PROTEFLAZID®, the proliferative activity of cells was significantly reduced and the integration of HPV DNA into the genome of cervical epithelial cells was blocked, which indirectly indicated stabilization of the cellular genome and restoration of proto-oncogene control over the cell cycle.

Even though the concept of the viral nature of precancerous diseases and cervical cancer has been finally formed, the place of conservative antiviral therapy in HPV-infected women is not clearly defined in the regulatory documents on the management of patients with HPV-associated CIN.

Conclusions

The results of our clinical and molecular-morphological studies have shown that the simultaneous use of the domestic drug PROTEFLAZID® (drops) and PROTEFLAZID® (suppositories) for 3 months in HPV-infected women without signs of morphological damage to the cervix contributed to the eradication of HPV in 86.7% of cases, and in women with CIN I caused by HPV – in 76.7% of cases; at the same time, the treatment contributed to the regression of cervical dysplasia in 76.7% of women. It can also be stated that the therapy of HPV infection with the use of the drug PROTEFLAZID® in women with CIN I leads to a stable regression of dysplastic lesions of the cervical epithelium, since during the 24-month follow-up after treatment, all PAP and HPV tests in the studied women were negative.

The results of the study once again confirmed the need for antiviral treatment in women at the initial stage of HPV infection, which is manifested by koilocytosis in cytological preparations, that is, until the moment when HPV can cause CIN I or more severe dysplastic lesions of the cervix. Such treatment can be attributed, like vaccination, to the primary prevention of cervical cancer, since it has been proven that women in the absence of HPV infection have an extremely low risk of developing cervical cancer.

Therefore, PROTEFLAZID® is currently one of the most effective drugs for the treatment of HPV infection, which can be recommended for the eradication of papillomaviruses

in women, both with low-risk cervical intraepithelial neoplasia (CIN I), and with a positive HPV test and with a normal PAP test.

References:

1. Багній М. М., Діброва В. А., Попадинець О. Г., Гришук М. І. Методики морфологічних досліджень. Вінниця: Нова Книга, 2016. – С. 328.
2. Волошина Н. М., Званцева Є. Д. Шляхи зниження діагностичної та лікувальної агресії у пацієнток з ВПЛ-інфекцією у репродуктивному віці // Здоров'я жінки, 2017. – №9 (125).
3. Воробйова Л. І., Лігірда Н. Ф. Порівняння терапевтичної ефективності застосування різних лікарських форм препарату альфа-2-інтерферону у хворих на ЦІН I та ЦІН II //Здоров'я жінки, 2010. – №5 (51). – 231–236.
4. Захарцева Л. М., Манжура Є. П., Вакуленко Г. В. та ін. Гіперекспресія білка p16 в епітеліальних клітинах шийки матки та його значення в оцінці ризику формування раку // Здоров'я жінки, 2011. – №4 (57). – С. 253–256.
5. Годлевська Н. А., Стровер В. В. Клінічна ефективність місцевого застосування вагінальних супозиторіїв Протефлазід у лікуванні цервікальної інтраепітеліальної неоплазії легкого та помірного ступенів, зумовленої вірусом папіломи людини // Здоров'я жінки, 2017. – №2 (118).
6. Жук С. І., Кошмеринська О. М., Таран О. О. Цервікальна інтраепітеліальна неоплазія – актуальна проблема сьогодення (огляд літератури) // Неонатологія, хірургія та перинатальна медицина, 2012. – №4 (6). – С.207–213.
7. Камінський В. В., Шалько М. Г., Гриневич О. Й. Протефлазід у вагітних: системний огляд результатів післяреєстраційних спостережень за безпечністю та ефективністю застосування // Здоров'я жінки, 2014. – №6 (92).
8. Камінський В. В., Шалько М. Г. Нові терапевтичні підходи до лікування жінок з дисплазією епітелію шийки матки, зумовленою папіломавірусною інфекцією //Інформаційний лист про нововведення в системі охорони здоров'я. – К., 2016 №49.
9. Кравчук І. В. Експресія маркерів p16, Ki67 та p63 у жінок із цервікальною патологією на тлі інфікування вірусом папіломи людини високого канцерогенного ризику // Здоров'я жінки, 2009. – №8 (44). – С. 233–216.
10. Лакатош В. П. Діагностика та лікування цервікальних інтраепітеліальних неоплазій папіломавірусної етіології з використанням клінічних, цитоморфологічних, ультраструктурних та молекулярно-біологічних критеріїв //Доктор, 2006. – №5. – С. 51–55.
11. Волошина Н. М., Кузнецова Т. П., Паук О. П. та інші. Лікування захворювань шийки матки, що асоціюються з папіломавірусною інфекцією у пацієнток молодого віку // Таврійський медико-біологічний вісник, 2011. – №3, ч. 2(55). – С. 56–60.
12. Національний консенсус щодо ведення пацієнток з цервікальними інтраепітеліальними неоплазіями, зумовленими папіломавірусною інфекцією (2017 р.).
13. Пітько В. А., Гузь І. А., Демиденко О. Д. та співавт. Папіломавірусна інфекція: лікування та профілактика // Таврійський медико-біологічний вісник, 2011. – Т.14, №3, ч. 2 (55). – С. 158–160.
14. Запорожан В. М., Татарчук Т. Ф., Дубініна В. Г. та ін. Передпухлинна патологія шийки матки: обсяг компетенції лікаря-гінеколога // Репродуктивна ендокринологія, 2013. – №7. – С.7–15.
15. Камінський В. В., Шалько М. Г., Воробйова Л. І. та інші. Протефлазід: специфічна активність у доклінічних дослідженнях; ефективність та безпечність застосування у клінічній практиці при захворюваннях, спричинених вірусом папіломи людини (систематичний огляд) //Здоров'я жінки, 2015. – №3 (99). – С.122–132.

16. Свінцицький В. С., Приймак В. В., Ренкас О. П. Сучасні підходи до діагностики та лікування раку шийки матки /Укр. мед. журнал, 2020, т. 2. – С. 1–7.
17. Суханова А. А. Сучасні погляди на роль інфекційного фактора у виникненні та розвитку дисплазії епітелію шийки матки // Вісник асоціації акушерів-гінекологів України, 1999. – №2. – С.9–14.
18. Татарчук Т. Ф., Сіліна Н. К. Сучасна тактика ведення пацієнток із ВПЛ-асоційованими неоплазіями. Основні рекомендації EUROGIN 2011 // Гінекологічна ендокринологія, 2011. – №1. – С. 78–80.
19. Бенюк В. О., Гриневич О. Й., Усевич І. А. та ін. Терапевтична ефективність супозиторіїв Протекфлазид при змішаній урогенітальній вірусно-бактеріальній інфекції // Здоров'я жінки, 2016. – №2 (108).
20. Український національний консенсус щодо обстеження та лікування жінок після проходження первинного цервікального скринінгу (2022 р.).
21. Уніфікований клінічний протокол первинної, вторинної (спеціалізованої), третинної (високоспеціалізованої) медичної допомоги «Дисплазія шийки матки. Рак шийки матки» (Наказ МОЗ України №236 від 02.04.2014 р.).
22. Шпонька І. С., Дорохова О. В., Шпонька О. В. Прогностичне та діагностичне значення експресії маркерів біологічних властивостей тканин при диспластичних та неопластичних процесах шийки матки // Медичні перспективи, 2005. – Том X, 4. – С. 8–16.
23. Шпонька О. В. Діагностика, прогноз та лікування дисплазії шийки матки, асоційованої з папіломавірусною інфекцією у жінок репродуктивного віку. Дис. к. мед. н., 2012.
24. Allanson E. R., Schmeler K. M. Preventing Cervical Cancer Globally: Are We Making Progress? / Cancer Prev Res (Phila), 2021. 14 (12): 1055–1060.
25. Bogani G., Chiappa V., Pinelli C. et al. Trends in prevalence in human papillomavirus types and their association with cervical dysplasia: an analysis on 15 138 women over 20 years. / Eur. J. Cancer Prev., 2020. 29(5): 452–457.
26. Isidean S. D., Wang Y., Mayrand M. H. et al. Assessing the time dependence of prognostic values of cytology and human papillomavirus testing in cervical cancer screening. Int. J. Cancer, 2019. 144 (10): 2408–2418. DOI: 10.1002/ijc.31970.
27. Ntanasis-Stathopoulos I., Kyriazoglou A., Liontos M. A. et al. () Current trends in the management and prevention of human papillomavirus (HPV) infection. / J. BUON, 2020. 25 (3): 1281–1285.
28. Okunade K. S. Human papillomavirus and cervical cancer. / J. Obstet. Gynaecol., 2020, 40 (5): 602–608.
29. World Health Organization. WHO Guidelines for Treatment of Cervical Intraepithelial Neoplasia 2–3 and Adenocarcinoma in situ: Cryotherapy, Large Loop Excision of the Transformation Zone, and Cold Knife Conization. Geneva. WHO (2014).



Section 4

4.1. PROTEFLAZID®: TREATMENT OF HERPESVIRUS AND MIXED INFECTIONS. META-ANALYSIS OF CLINICAL TRIALS RESULTS

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Abstract

There is a meta-analysis of clinical trials results concerning the evaluation of efficiency of the drug PROTEFLAZID® (drops) in the treatment of adult patients with herpesvirus and mixed infections.

Objective of the study: to evaluate the clinical efficacy of the drug PROTEFLAZID® in a treatment of herpesvirus (HSV-1, HSV-2) and mixed infections in adults by meta-analysis of clinical trials.

Materials and research methods. In meta-analysis 16 scientific sources with results of controlled studies with participation of 1336 patients over 18 y.o. during 2003-2015 are included.

Results. Obtained data are indicative of efficiency of the drug PROTEFLAZID® in the treatment of patients with herpesvirus and mixed infections. PROTEFLAZID® induces improvement in the main clinical signs of diseases, promotes elimination of herpesviruses from organism and prevention of relapses.

Conclusions. PROTEFLAZID® in the therapy of various clinical forms of HHVs infection promotes the improvement in the main clinical signs of diseases, elimination of HSV-1 and HSV-2; prevention of relapses of diseases (ophthalmoherpes, genital herpes) after completion of the treatment. Therapy of mixed infections (HSV, bacteria, protozoa, fungi) using the drug PROTEFLAZID® promotes improvement in the main clinical signs of diseases, improvement in vaginal microflora condition and improvement in local changes in the cervix in women with inflammatory diseases of genital organs as well as in prevention of relapses of genital inflammatory diseases and chronic pyelonephritis.

Key words: human herpesviruses infection, mixed infection, PROTEFLAZID®, clinical trials, meta-analysis.

Introduction

Specific characteristic of the modern infectious pathology is an incidence rate of chronic infectious-inflammatory diseases that form quite enough against the background of persistent human herpesviruses (HHV) infection and mixed infections (herpesviruses in various possible combinations with other microorganisms: bacteria, protozoa, fungi).

Herpesviruses cause a wide variety of clinical forms of infections depending on the site of the pathological process and its prevalence, the condition of the patient's immune system.

HHV infection is caused most often by herpes simplex virus (HSV) – HSV-1 and HSV-2 – antibodies to which are detected in 90-99% of the adult population of the planet. HSV-1 infection usually occurs in the first 3 years of a child's life, and HSV-2 during puberty. According to the WHO, diseases caused by HSV, with regard to the fatality rate due to viral infections, take the

second place (15.8%) after the flu (35.8%). Literature data indicate that about 80% of the population is infected by HSV-1, and 10% of women and 15% of men by HSV-2. Ophthalmologists register herpetic keratitis and iridocyclitis in 60% of cases of corneal lesions [28].

HHV infection runs both in the form of a mono- and mixed infection, and in association with viral, bacterial and other infectious agents (mixed infection) which contributes to the potentiation of their pathogenic properties. The leading role of the association of HSV-2 and human papilloma virus in the development of cervical cancer, and prostate cancer has been proved. Association of HSV-2, CMV and toxoplasma has a negative effect on the course of pregnancy and childbirth [12].

The main biological and pathogenetic features of herpesviruses are their lifelong persistence in the body of a person once infected by them, the strongest dependence of the course of the chronic process on the state of the virus carrier immunity and the tendency to recur.

The lifelong persistence of herpesviruses in the human body is due to their unique ability to incorporate their DNAs into the neurons of the regional (in relation to external infection site) ganglia of the sensory nerves of the peripheral nervous system. This way of preserving the parasitic genome in the human body leads to the fact that during the off-recessive period of herpetic infection the immune system that always recognizes only biologically foreign antigens circulating in the body and does not have access to the intracellular spaces "does not see" the DNAs of the herpesviruses and does not react to them, and hence the problem of "detecting" and studying of herpesviruses remains actual [19].

Abnormalities of the immune system play an important role in the persistence of the virus and in the pathogenesis of recurrent herpes. If the immune status is compromised, the herpetic infection becomes more severe with frequent and prolonged relapses which is due to the lack of immunity or an excessive immune response. In turn, herpetic infection causes immunosuppression [21].

The issue of the effective therapy of herpesvirus infection remains in the center of attention of physicians of different specialties due to the annual increase in the number of patients with severe disease and frequent transition to a chronic process. Modern medicine does not have treatment methods that completely eliminate viruses of herpes from the human body. The issue of resistance of viruses to traditional antiviral therapy is also crucial. Presently, effective and safe drugs for the long-term use thereof are sought [15].

One of the most promising agents showing a pronounced direct antiviral effect on DNA-containing viruses, to which the group of herpes viruses belongs, is the proprietary drug Proteflazid[®] widely used in the clinical practice (SMC Ecopharm Ltd., Ukraine), the active substance of which is obtained from plants *Deschampsia caespitosa* L. and *Calamagrostis epigeios* L. The main biologically active component of the active substance of the drug is a complex compound of O- and C-glycoside flavones: tricetin, luteolin, and apigenin. The drug Proteflazid[®] is characterized by a number of basic pharmacodynamic properties: etiotropic action: it suppresses virus-specific enzymes of DNA- and RNA-virus synthesis (thymidine kinase, DNA and RNA polymerase, reverse transcriptase) which is accompanied by a decrease in the ability of replication of viral nucleic acids or complete blockade thereof and, as a result, makes it impossible to duplicate DNA and RNA viruses; pathogenetic action: it has an immunotropic mechanism of action – optimizes the synthesis of endogenous α - and β -interferons (IFNs), restores tissue immunity by increasing the level of secretory immunoglobulin A (sIgA); has an antioxidant mechanism of action – it helps to reduce the activity of lipid peroxidation and increase the activity of antioxidant protection resulting in reduced endotoxemia and increased resistance of cells and tissues to the damaging effects of free radical oxidation products whose level in the body is increased in most pathological processes, especially in inflammation and infectious damage [14,15,28].

The polypharmacological action of the drug Proteflazid® consisting of direct antiviral, immunotropic and antioxidant actions simultaneously provides etiotropic and pathogenetic effects on both viral and mix infections including undesirable processes in the human body caused by this infection. The above properties of the drug Proteflazid® give rise to its wide application in various fields of medicine for the prevention and treatment of a number of infectious diseases caused by viruses: HSV-1, HSV-2, Epstein- Barr virus (EBV), cytomegalovirus (CMV), human papillomavirus, hepatitis, HIV, influenza and ARVI, as well as in the complex therapy of viral-bacterial and fungal infections [1-4,6,14,17, 20].

In clinical studies, it has been shown that, when applied topically, the drug Proteflazid® (suppositories or vaginal tampons with drug solution) restores the protective function of the vaginal and cervical mucosa due to normalization of tissue immunity factors (sIgA, lysozyme and complement component C₃), promotes rapid and effective elimination of the causative agent [2,6,17]. The drug Proteflazid® prevents relapses of the disease and prolongs the period of remission [1,20].

The effective and safe use of the drug Proteflazid® (drops) in the treatment of HHV infection is shown in a number of published meta-analysis and systematic reviews of clinical studies [1,3,4,14,20].

Objective of the study

To evaluate the clinical efficacy of the drug Proteflazid® in the treatment of herpesvirus (HSV-1, HSV-2) and mixed infections in adults by meta-analysis of the clinical studies.

Materials and methods

Information on clinical studies regarding the effectiveness of the use of the drug Proteflazid® in the treatment of HHV infection (mono- and mixed infections) in adult patients was obtained using information retrieval systems on the Internet. In addition, the developer of the drug Proteflazid® (SMC Ecopharm Ltd., Ukraine) provided some reports on the clinical trial results of the drug in the treatment of HHV infection.

At the initial stage of the selection, 23 scientific sources with the results on clinical trials of the effectiveness of the drug Proteflazid® in adult patients with HHV infection (mono- and mixed infections) were identified. In accordance with the inclusion criteria, 13 publications and 3 clinical reports on the results of controlled clinical trials with regard to effectiveness of the drug Proteflazid® in the treatment of HHV infection and mixed infections involving 1336 patients over 18 y.o. during 2003-2015 were selected for metaanalysis, 7 publications were excluded from meta-analysis.

The list of publications selected and included in meta-analysis is presented in the tab. 1.

Table 1. The list of selected publications reflecting the effectiveness of the use of the drug Proteflazid® in the treatment of HHV infection and mixed infections in adult patients

Author	Year	Total number of patients	Application field of the drug Proteflazid®	Clinical trials results
1. Publications reflecting the effectiveness of the use of the drug Proteflazid® in the treatment of HHV infection caused by HSV-1 and HSV-2 in adults				
Petrunya A.M. [24]	2003	47	Keratitis caused by herpes-virus infection	Positive dynamics of clinical indicators, prolongation of disease-free survival, reduction of relapse rate

Lesovoy V.N. [19]	2006	54	Genital herpes	Significant reduction in viral replication, normalization of immunological parameters
Rykov S.O. [26]	2010	134	Keratitis caused by herpesvirus infection	Stabilization of the infectious process, improvement in visual functions, reduction of relapse rate
Klymenko P.M. [15]	2012	40	Urogenital herpes	Improving the effectiveness of therapy, acceleration of the onset of the remission phase, reduction of relapse rate
Kornatskaya A.G. [16]	2015	30	Genital herpes	Significant reduction in clinical manifestations of the disease, reduction of relapse rate
Hopchuk O.M. [9]	2006	70	Genital herpes	A positive therapeutic effect, reduction of relapse rate
Sundukov A.V. [30]	2008	52	Genital herpes	Significant reduction in clinical manifestations of the disease, reduction of relapse rate, prolongation of inter-relapse period
Zapolskiy M.E. [12]	2012	88	Multiform exudative erythema associated with herpesvirus infection	Improving the function of the immune system, reduction of relapse rate
2. Publications reflecting the effectiveness of the use of the drug Proteflazid® in the treatment of mixed infections in adults				
Romashchenko O.V. [27]	2005	60	Inflammatory diseases caused by mixed infection	Increasing the effectiveness of therapy, reduction of disease of genitals in women relapse rate
Rak L.M. [25]	2013	64	Inflammatory diseases of uterine adnexa caused by mixed infection	Increasing the effectiveness of therapy, reduction of disease relapse rate
Beniuk V.A. [7]	2015	70	Urogenital mixed infection	Significant reduction in clinical manifestations of the disease, reduction of relapse rate
Sidorenko E.V. [29]	2010	195	Chronic pyelonephritis caused by mixed infection	Increasing the effectiveness of complex therapy, reduction of disease relapse rate
Kolesnik M.O. [18]	2014	150	Acute and chronic pyelonephritis caused by mixed infection	Increasing the effectiveness of complex therapy, reduction of disease relapse rate
Shvedyuk S.V. [32]	2003	22	Urogenital clamidiosis coupled with genital herpes	Increasing the effectiveness of therapy, reduction of disease relapse rate

Grinkevich T.M. [11]	2005	120	Genital herpes	Significant reduction in clinical manifestations of the disease, reduction of relapse rate
Bayev A.I. [5]	2011	140	Mixed-STIs	Clinical improvement, normalization of immunological and biochemical parameters

Software

To ensure a higher degree of reliability of the results two specialized programs RevMan and SPSS were used.

Inclusion criteria

Studies that were included in meta-analysis corresponded to the following criteria:

1. Controlled studies provided control or control group and focused on examination of the clinical efficacy of the drug Proteflazid® in the treatment of HHV infection (HSV-1, HSV-2) and mixed infections (HSV, bacteria, protozoa, fungi) in patients over 18 y.o.

2. Mandatory confirmation of clinical diagnosis.

3. Published data on the evaluation of the effectiveness of the drug Proteflazid® are complete.

Studies that did not meet the stated criteria were excluded from meta-analysis.

All patients before the beginning of the treatment and after completion of therapy were thoroughly examined including the generally accepted clinical and laboratory studies, ultrasound, the study of the parameters of cellular and humoral immunity. As the main diagnostic method, the methods of enzyme immunoassay (ELISA) and polymerase chain reaction (PCR) were used.

Meta-analysis of clinical studies was carried out for the following statistically significant indicators:

1. Dynamics of clinical symptoms and manifestations of HHV infection in the adult patients against the background of treatment using the drug Proteflazid®:

- incidence of positive dynamics of clinical symptoms in patients with HHV infection;
- incidence of elimination of HSV-1 and HSV-2 detected using PCR in patients with HHV infection;

2. Dynamics of clinical symptoms and manifestations of mixed infections (HSV, bacteria, protozoa, fungi) in the adult patients against the background of treatment using the drug Proteflazid®:

- incidence of positive effect of the treatment of inflammatory diseases of genitals caused by a mixed infection;
- incidence of improvement in the condition of the vaginal microflora in women with inflammatory diseases of the genitals caused by a mixed infection;
- incidence of positive dynamics of local changes in the cervix in women with inflammatory diseases of the genitals caused by a mixed infection.

3. Prevention of the disease relapse after completion of the therapy:

- incidence of ophthalmoherpes relapse;
- incidence of genital herpes relapse;
- incidence of relapse of chronic pyelonephritis caused by mixed infection;
- incidence of relapse of inflammatory diseases of genitals caused by mixed infection.

Clinical test results according to said indicators are provided in tables 2-10.

Table 2. Incidence of positive dynamics of clinical symptoms in patients with HHV infection

Author	Main group		Control group	
	Number of patients treated with Proteflazid® who had a positive dynamics of clinical symptoms	Total number of patients	Number of patients who didn't take Proteflazid® and had a positive dynamics of clinical symptoms	Total number of patients
Shvedyuk S.V., 2003	9	11	8	11
Hopchuk O.M., 2006	29	35	20	35
Lesovoy V.N., 2006	27	30	17	24
Klymenko P.M., 2012	19	30	5	10
Zapolskiy M.E., 2012	31	32	16	26
Kornatskaya A.G., 2015	35	35	35	35
Total	150	173	101	141

According to the indicator “The incidence of positive dynamics of clinical symptoms in patients with HHV infection” against the background of the drug Proteflazid®, 314 patients were examined of which 173 were the main group, and 141 constituted the control group (Table 2).

Table 3. Incidence of elimination of HSV-1 and HSV-2 detected using PCR in patients with HHV infection

Author	Main group		Control group	
	Number of patients treated with Proteflazid® who had a marked elimination of HSV-1 and HSV-2	Total number of patients	Number of patients who didn't take Proteflazid® and had a marked elimination of HSV-1 and HSV-2	Total number of patients
Hopchuk O.M., 2006	29	35	20	35
Lesovoy V.N., 2006	27	30	17	24
Total	56	65	37	59

According to the indicator “The incidence of elimination of HSV-1 and HSV-2 detected using PRC in patients with HHV infection” against the background of the drug Proteflazid®, 124 patients with HHV infection were examined of which 65 were the main group, and 59 constituted the control group (Table 3).

Table 4. Incidence of positive effect of the treatment of inflammatory diseases of genitals caused by a mixed infection

Author	Main group		Control group	
	Number of patients treated with Proteflazid® who had a positive effect of the treatment	Total number of patients	Number of patients who didn't take Proteflazid® and had a positive effect of the treatment	Total number of patients
Romashchenko O.V., 2005	25	30	19	30
Bayev A.I., 2011	72	80	43	60

Rak L.M., 2013	28	32	21	32
Beniuk V.A., 2015	34	35	33	35
Total	159	177	116	157

According to the indicator “The incidence of positive effect of the treatment of inflammatory diseases of genitals caused by a mixed infection” against the background of the drug Proteflazid®, 334 patients with inflammatory diseases of genitals caused by mixed infection were examined of which 177 were the main group, and 157 constituted the control group (Table 4).

Table 5. Incidence of improvement in the vaginal microflora condition in women with inflammatory diseases of the genitals caused by a mixed infection

Author	Main group		Control group	
	Number of female patients treated with Proteflazid® who had a marked improvement in vaginal microflora condition	Total number of female patients	Number of female patients who didn't take Proteflazid® and had a marked improvement in vaginal microflora condition	Total number of female patients
Romashchenko O.V., 2005	25	30	18	30
Hopchuk O.M., 2006	29	35	20	35
Rak L.M., 2013	27	32	19	32
Total	81	97	57	97

According to the indicator “The incidence of cases of improvement in the vaginal microflora condition in women with inflammatory diseases of the genitals caused by a mixed infection” against the background of the drug Proteflazid®, 194 female patients with inflammatory diseases of the genitals caused by a mixed infection were examined of which 97 were the main group, and 97 constituted the control group (Table 5).

Table 6. Incidence of positive dynamics of the local changes in the cervix in women with inflammatory diseases of the genitals caused by a mixed infection

Author	Main group		Control group	
	Number of female patients treated with Proteflazid® who had a positive dynamics of local changes in the cervix	Total number of female patients	Number of female patients who didn't take Proteflazid® and had a positive dynamics of local changes in the cervix	Total number of female patients
Romashchenko O.V., 2005	6	30	2	30
Rak L.M., 2013	18	32	3	32
Total	24	62	5	62

According to the indicator “The incidence of cases of positive dynamics of local changes in the cervix in women with inflammatory diseases of the genitals caused by a mixed infection” against the background of the drug Proteflazid®, 124 female patients with inflammatory diseases

of the genitals caused by a mixed infection were examined of which 62 were the main group, and 62 constituted the control group (Table 6).

Table 7. Incidence of ophthalmoherpes relapse

Author	Main group		Control group	
	Number of patients treated with Proteflazid® who had ophthalmoherpes relapse	Total number of patients	Number of patients who didn't take Proteflazid® and had ophthalmoherpes relapse	Total number of patients
Petrunya A.M., 2003	2	22	5	25
Rykov S.O., 2010	4	50	22	50
Total	6	72	27	75

According to the indicator “The incidence of ophthalmoherpes relapse” against the background of the drug Proteflazid®, 147 patients with herpes simplex keratitis and uveitis were examined of which 72 were the main group, and 75 constituted the control group (Table 7).

Table 8. Incidence of genital herpes relapse

Author	Main group		Control group	
	Number of patients treated with Proteflazid® who had genital herpes relapse	Total number of patients	Number of patients who didn't take Proteflazid® and had genital herpes relapse	Total number of patients
Shvedyuk S.V., 2003	2	11	5	11
Grinkevich T.M., 2005	39	49	45	51
Sundukov A.V., 2008	10	22	24	30
Zapolskiy M.E., 2012	4	32	9	26
Klymenko P.M., 2012	2	30	3	10
Total	57	144	86	128

According to the indicator “The incidence of genital herpes relapse” against the background of the drug Proteflazid®, 272 patients with genital herpes were examined of which 144 were the main group, and 128 constituted the control group (Table 8).

Table 9. Incidence of relapse of chronic pyelonephritis caused by mixed infection

Author	Main group		Control group	
	Number of patients treated with Proteflazid® who had chronic pyelonephritis relapse	Total number of patients	Number of patients who didn't take Proteflazid® and had chronic pyelonephritis relapse	Total number of patients
Sidorenko E.V., 2010	14	85	33	110
Kolesnik M.O., 2014	0	60	6	90
Total	14	145	39	200

According to the indicator “The incidence of relapse of chronic pyelonephritis caused by mixed infection” against the background of the drug Proteflazid®, 345 patients with chronic pyelonephritis were examined of which 145 were the main group, and 200 constituted the control group (Table 9).

Table 10. Incidence of relapse of inflammatory diseases of genitals caused by mixed infection

Author	Main group		Control group	
	Number of patients treated with Proteflazid® who had relapse of inflammatory diseases of genitals	Total number of patients	Number of patients who didn't take Proteflazid® and had relapse of inflammatory diseases of genitals	Total number of patients
Romashchenko O.V., 2005	1	30	5	30
Rak L.M., 2013	1	32	5	32
Total	2	62	10	62

According to the indicator “The incidence of relapse of inflammatory diseases of genitals caused by mixed infection” against the background of the drug Proteflazid®, 124 patients with inflammatory diseases of genitals caused by mixed infection were examined of which 62 were the main group, and 62 constituted the control group (Table 10).

Research results and discussion thereof

Dynamics of clinical symptoms and manifestations of HHV infection in the adult patients on the background of the treatment using drug Proteflazid®.

Fig. 1 depicts the Forest plot of the results of meta-analysis based on identification of the odds ratio of the incidence of positive dynamics of clinical symptoms in patients with HHV infection after use of the drug Proteflazid® in the main and control groups.

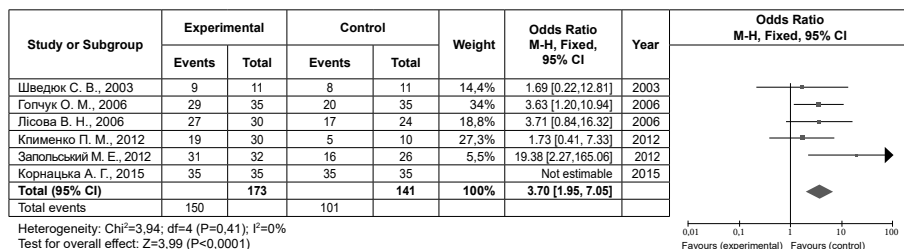


Figure 1. Results of meta-analysis based on identification of the odds ratio of the incidence of positive dynamics of clinical symptoms in patients with HHV infection

The value of -square test (P=0.41) and I²-test (I²=0%) prove the homogeneity of these studies, therefore a model with a fixed effect is selected. P-value of Fisher's ratio test (P = 0.0001) shows significance of the selected effect.

The value of odds ratio (OR = 3.70) indicates that the probability of an incidence of positive dynamics of clinical symptoms in patients with HHV infection after use of the drug Proteflazid® in the main group is 3.70 times higher than in the control group.

According to this indicator, the most significant is the study by Gopchuk O.M. (2006) which states that the use of the drug Proteflazid® in the complex treatment of herpes-virus genital lesions is more clinically, microbiologically and immunologically effective than traditional methods [9]. A study conducted by Zapolsky M.E. (2012) notes that the use of the drug Proteflazid® contributes to a significant decrease in the CIC content ($p<0.05$), an increase in IgG [12]. Kornatskaya A.G. (2015) concludes that the use of the drug Proteflazid® (vaginal tampons with drug solution) promotes an increase in the level of tissue immunity indices (secretory IgA, lysozyme, complement component C₃) [16].

Fig. 2 shows the Forest plot of the results of meta-analysis based on the identification of the odds ratio of the incidence of elimination of HSV-1 and HSV-2 detected by PCR in patients with HHV infection after use of the drug Proteflazid® in the main and control groups.

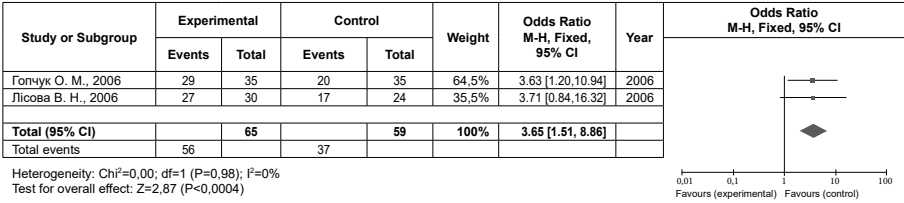


Figure 2. Results of meta-analysis based on the identification of the odds ratio of the incidence of elimination of HSV-1 and HSV-2 detected by PCR in patients with HHV infection

The value of -square test (P=0.98) and I²-test (I²=0%) prove the homogeneity of these studies, therefore a model with a fixed effect is selected. P-value of Fisher’s ratio test (P = 0.004) shows significance of the selected effect.

The value of odds ratio (OR=3.65) indicates that the probability of an incidence of elimination of HSV-1 and HSV-2 detected by PCR in patients with HHV infection after using of the drug Proteflazid® in the main group is 3.65 times higher than in the control group.

The most significant is the research by Gopchuk O.M. (2006) which states that therapy with the inclusion of the drug Proteflazid® contributes to a reliable increase in the number of CD4 + lymphocytes and NK cells to normal values, an increase in the amount of IFN- and IFN- [9]. A study conducted by Lesovoy V.N. et al. (2006) notes that the drug Proteflazid® increases the positive effect of the therapy by 15-20% [19].

Dynamics of clinical symptoms and manifestations of mixed infection (HSV, bacteria, protozoa, fungi) in the adult patients on the background of the treatment using drug Proteflazid®.

Figure 3 depicts the Forest plot of the results of meta-analysis based on identification of the odds ratio of the incidence of positive effect of the treatment of inflammatory diseases of genitals caused by mixed infection after use of the drug Proteflazid®

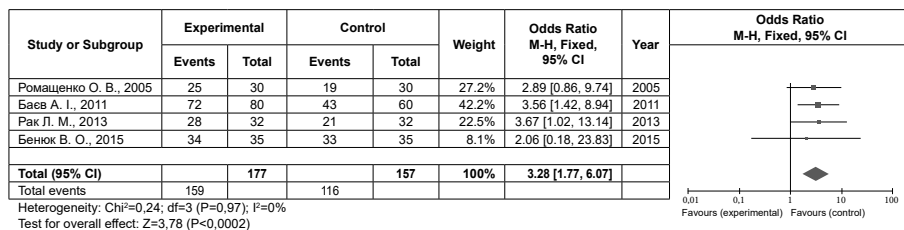


Figure 3. Results of meta-analysis based on identification of the odds ratio of the incidence of positive effect of the treatment of inflammatory diseases of genitals caused by mixed infection

The value of χ -square test ($P=0.97$) and I^2 -test ($I^2=0\%$) prove the homogeneity of these studies, therefore a model with a fixed effect is selected. P-value of Fisher's ratio test ($P=0.0002$) shows significance of the selected effect. The value of odds ratio ($OR=3.28$) indicates that the probability of an incidence of positive effect of the treatment of inflammatory diseases of genitals caused by mixed infection after use of the drug Proteflazid® in the main group is 3.28 times higher than in the control group.

The most significant is the study by Baev A.I. et al. (2011) who indicate the most significant clinical and immunological changes in the use of the drug Proteflazid®: an increase in the relative percentage of CD3, CD4, CD20-lymphocytes; decrease in CD8-lymphocyte content and CD4/CD8 ratio; normalization of the indices of the main classes of immunoglobulins A, M and G [5].

Benyuk V.A. (2015) notes that the evidence of the effectiveness of the therapy with the use of the drug Proteflazid® in both tested groups was a reliable increase in tissue immunity indices. In particular, the level of secretory IgA and the level of lysozyme increased by the 14th day of the treatment remaining significantly high throughout the follow-up period (for secretory IgA: 1641.9 $\mu\text{g/L}$ at the screening, 2154.2 $\mu\text{g/L}$ on the 14th day, 2859.3 $\mu\text{g/L}$ at the end of the 4-week observation, for the lysozyme: 26.7 $\mu\text{g/L}$ at the screening, 48.2 $\mu\text{g/L}$ on the 14th day, 39.0 $\mu\text{g/L}$ at the end of the 4-week observation); level of the complement component C_3 increased in the main group by the 14th day of the treatment and returned to baseline by the end of the 4-week observation period (24.7 $\mu\text{g/g}$ protein at the screening, 33.0 $\mu\text{g/g}$ protein on the 14th day, 24.5 $\mu\text{g/g}$ protein at the end of the 4-week observation for the complement component C_3) [7].

Figure 4 shows the Forest plot of the results of meta-analysis based on the identification of the odds ratio of the incidence of improvement in condition of vaginal microflora in women with inflammatory diseases of genitals caused by mixed infection after use of the drug Proteflazid® in the main and control groups.

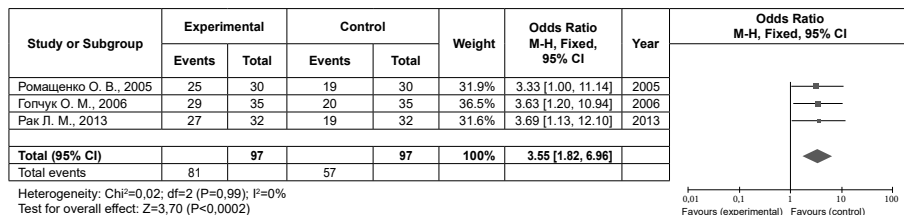


Figure 4. Results of meta-analysis based on the identification of the odds ratio of the incidence of improvement in condition of vaginal microflora in women with inflammatory diseases of genitals caused by mixed infection

The value of χ -square test ($P=0.99$) and I^2 -test ($I^2=0\%$) prove the homogeneity of these studies, therefore a model with a fixed effect is selected. P-value of Fisher’s ratio test ($P = 0.0002$) shows significance of the selected effect.

The value of odds ratio ($OR=3.55$) indicates that the probability of an incidence of improvement in vaginal microflora condition in wo men with inflammatory diseases of genitals caused by mixed infection after use of the drug Proteflazid® in the main group is 3.55 times higher than in the control group.

The most significant is the research by Gopchuk O.M. (2006) which notes that the inclusion of the drug Proteflazid® in the complex therapy of herpetic infection in women contributes to the normalization of the vaginal microbiocenosis [9].

Figure 5 shows the Forest plot of the results of meta-analysis based on the identification of the odds ratio of the incidence of positive dynamics of the local changes in the cervix in women with inflammatory diseases of genitals caused by mixed infection after use of the drug Proteflazid® in the main and control groups.

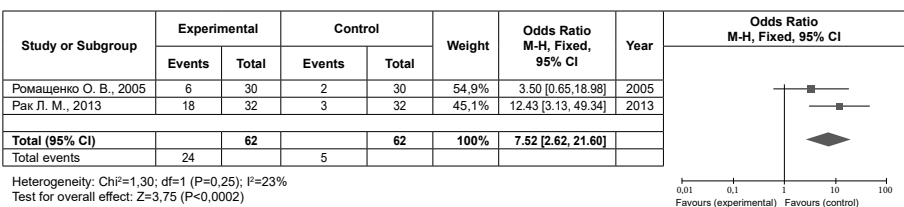


Figure 5. Results of meta-analysis based on the identification of the odds ratio of the incidence of positive dynamics of the local changes in the cervix in women with inflammatory diseases of genitals caused by mixed infection

The value of χ -square test ($P=0.25$) and I^2 -test ($I^2=23\%$) prove the homogeneity of these studies, therefore a model with a fixed effect is selected. P-value of Fisher’s ratio test ($P = 0.0002$) shows significance of the selected effect.

The value of odds ratio ($OR=7.52$) indicates that the probability of an incidence of positive dynamics of the local changes in the cervix in women with inflammatory diseases of genitals caused by mixed infection after use of the drug Proteflazid® in the main group is 7.52 times higher than in the control group. According to this indicator, the most significant is the study by (Romashchenko O.V. et al. who note that the use of the drug Proteflazid® was characterized by the development of marginal and mosaic epithelization of the erosive surface of the cervix by the end of treatment in 8 (26.2%) patients and complete epithelization in 6 (20%) patients [27].

Prevention of disease relapse after completion of the treatment

Figure 6 depicts the Forest plot of the results of meta-analysis based on identification of the odds ratio of the incidence of ophthalmoherpes relapse after use of the drug Proteflazid® in the main and control groups.

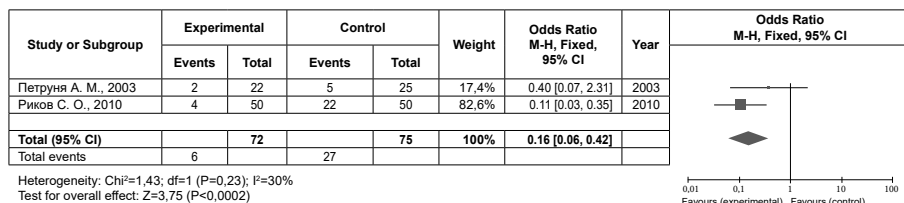


Figure 6. Results of meta-analysis based on identification of the odds ratio of the incidence of ophthalmoherpes relapse

The value of χ -square test ($P=0.23$) and I^2 -test ($I^2=30\%$) prove the homogeneity of these studies, therefore a model with a fixed effect is selected. P-value of Fisher's ratio test ($P = 0.0002$) shows significance of the selected effect.

The value of odds ratio ($OR=0.16$) indicates that the probability of an incidence of relapse of ophthalmoherpes (keratitis, uveitis) after use of the drug Proteflazid® in the main group is 6.25 times lower than in the control group.

The most significant is the study by Rykov S.O. et al. which proved that inclusion of antiviral and immunocorrecting drug Proteflazid® in the complex therapy for treatment of herpes simplex keratitis allows to decrease the duration of patients care (on average by 4-5 days) and decrease the incidence of relapses by 36% [26].

A study conducted by Petrunya A.M. et al. notes that the use of the drug Proteflazid® in a complex therapy and immunorehabilitation of patients with recidivating herpes simplex keratitis contributes to improving of clinical indices, reducing of the incidence of complications of said disease, increasing the duration of disease remission and reducing in the incidence of relapses. Authors conclude that the use of the drug Proteflazid® in patients with herpetic keratitis can be considered pathogenetically valid and reasonable [24].

Figure 7 depicts the Forest plot of the results of meta-analysis based on identification of the odds ratio of the incidence of genital herpes relapse after use of the drug Proteflazid® in the main and control groups.

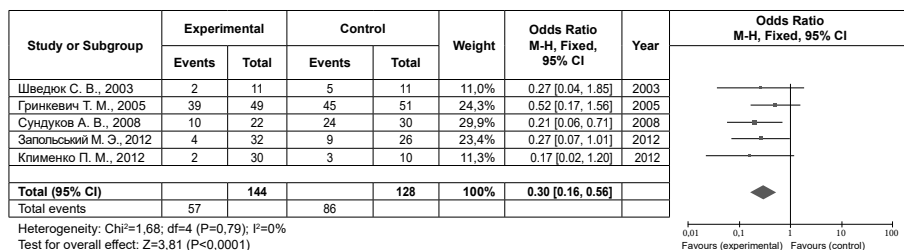


Figure 7. Results of meta-analysis based on identification of the odds ratio of the incidence of genital herpes relapse

The value of χ -square test ($P=0.79$) and I^2 -test ($I^2=0\%$) prove the homogeneity of these studies, therefore a model with a fixed effect is selected. P-value of Fisher's ratio test ($P = 0.0001$) shows significance of the selected effect.

The value of odds ratio (OR=0.30) indicates that the probability of an incidence of genital herpes relapse after use of the drug Proteflazid® in the main group is 3.33 times lower than in the control group.

The most significant is the study by Sundukov A.V. et al. who note the high efficacy of the drug Proteflazid® in the treatment of genital herpes that was expressed by significant reduction in the duration of the disease and a significant decrease in the number of relapses [30].

Shvedyuk S.V. et al. come to similar conclusions and note that the use of the drug Proteflazid® in patients with genital herpes has contributed to the regression of clinical symptoms of the disease, the normalization of the vascular pattern of the urethral mucosa, the purification of erosive- ulcerative defects, the disappearance sharp pain during urination [32].

A study by Klimenko P.M. et al. indicates that the inclusion of the drug Proteflazid® in the treatment regimen made it possible to shorten the genital herpes relapse in patients of the main group by 1.5 times as compared with control group and allowed to reduce the number of relapses by 4.5 times [15].

Figure 8 depicts the Forest plot of the results of meta-analysis based on identification of the odds ratio of the incidence of relapse of chronic pyelonephritis caused by infection after use of the drug Proteflazid® in the main and control groups.

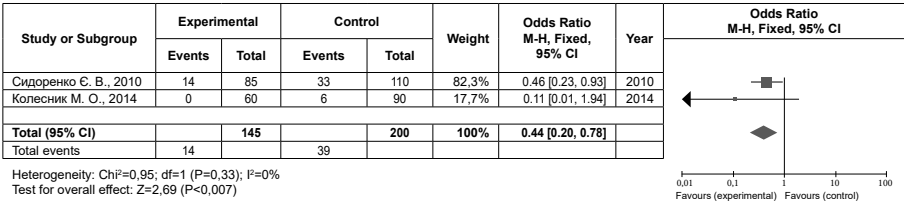


Figure 8. Results of meta-analysis based on identification of the odds ratio of the incidence of relapse of chronic pyelonephritis caused by infection

The value of χ -square test (P=0.33) and I²-test (I²=0%) prove the homogeneity of these studies, therefore a model with a fixed effect is selected. P-value of Fisher’s ratio test (P = 0.007) shows significance of the selected effect.

The value of odds ratio (OR=0.40) indicates that the probability of an incidence of relapse of chronic pyelonephritis caused by infection after use of the drug Proteflazid® in the main group is 2.5 times lower than in the control group.

The most significant is the research by Sidorenko E.V. that notes that the use of the drug Proteflazid® contributes to positive dynamics of clinical condition as well as functional activity of immunocompetent cells and condition of antioxidative system [29].

In their study, M. Kolesnik et al. consider that the drug Proteflazid® is a necessary component of the therapy for patients with chronic pyelonephritis, on the background of HHV infection, the use of which prevents the relapses of this disease [18].

Figure 9 depicts the Forest plot of the results of meta-analysis based on identification of the odds ratio of the incidence of relapse of inflammatory diseases of genitals caused by mixed infection after use of the drug Proteflazid® in the main and control groups.

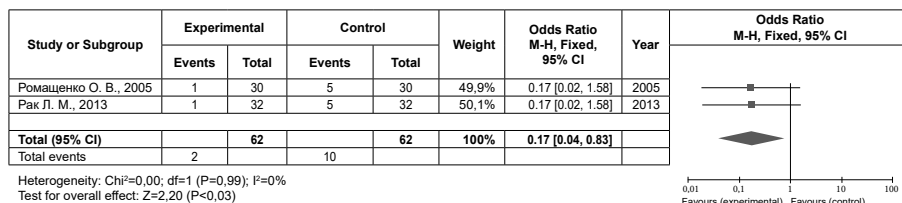


Figure 9. Results of meta-analysis based on identification of the odds ratio of the incidence of relapse of inflammatory diseases of genitals caused by mixed infection

The value of χ^2 -square test ($P=0.99$) and I^2 -test ($I^2=0\%$) prove the homogeneity of these studies, therefore a model with a fixed effect is selected. P-value of Fisher's ratio test ($P = 0.03$) shows significance of the selected effect.

The value of odds ratio ($OR=0.17$) indicates that the probability of an incidence of relapse of inflammatory diseases of genitals caused by mixed infection after use of the drug Proteflazid® in the main group is 5.88 times lower than in the control group.

The most significant are two initial studies. Rak L.M. et al. note that the incidence of relapse of inflammatory processes of uterine adnexa caused by mixed infection in female patients of control group is $15.6 \pm 0.5\%$, and in female patients that were administered the drug Proteflazid® is $3.1 \pm 0.7\%$ ($p < 0.01$) which confirms its clinical effectiveness and the advisability of using in a complex anti-inflammatory therapy in this category of female patients [25].

The study by Romashchenko O.V. et al. showed that inclusion of the drug Proteflazid® in the complex therapy of inflammatory diseases of genitals, on the background of HHV infection, in women accompanied with recovery to the normal levels of serum IFN titers, functional activity of phagocytes, indices of cell and humoral immunity 3 months after treatment completion. 6 months after treatment completion immunity indices in patients, in the scheme of therapy of which the drug Proteflazid® was included, did not differ from control [27].

Sensitivity analysis

The sensitivity analysis was carried out in such a way as to evaluate the impact of each individual study as for the combined data omitting an individual study. The results of the sensitivity analysis showed that no single study significantly affected the combined data indicating statistically reliable results.

Assessment of the bias of publications

Funnel plot was used to assess the bias of publications included in the study (fig. 10-18). Virtually all SE values in Figs. 10-18 are within the funnel which indicates that there is no systematic error.

Conducted meta-analysis allowed to broaden the evidence base regarding the efficacy of the drug Proteflazid® in the treatment of adult patients with herpesvirus (HSV-1, HSV-2) and mixed infections. 16 scientific sources with the results of clinical trials of the effectiveness of the drug Proteflazid® involving 1336 adult patients with HHV infection (mono- and mixed infections) during 2003-2015 were selected. Meta-analysis was conducted according to the following statistically significant criteria:

1. Dynamics of clinical symptoms and manifestations of HHV infection in the adult patients against the background of treatment using the drug Proteflazid®:
 - incidence of positive dynamics of clinical symptoms in patients with HHV infection;
 - incidence of elimination of HSV-1 and HSV-2 detected using PCR in patients with HHV infection.
2. Dynamics of clinical symptoms and manifestations of mixed infections (HSV, bacteria, protozoa, fungi) in the adult patients against the background of treatment using the drug Proteflazid®:
 - incidence of positive effect of the treatment of inflammatory diseases of genitals caused by a mixed infection;
 - incidence of improvement in the vaginal microflora condition in women with inflammatory diseases of the genitals caused by a mixed infection;
 - incidence of positive dynamics of local changes in the cervix in women with inflammatory diseases of the genitals caused by a mixed infection.
3. Prevention of the disease relapse after completion of the therapy:
 - incidence of ophthalmoherpes relapse;
 - incidence of genital herpes relapse;
 - incidence of relapse of chronic pyelonephritis caused by mixed infection;
 - incidence of relapse of inflammatory diseases of genitals caused by mixed infection.

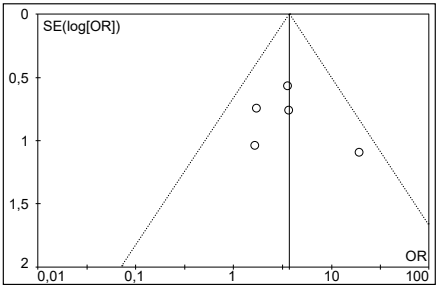


Figure 10. Funnel plot for the indicator “The incidence of positive dynamics of clinical symptoms in patients with HHV infection”

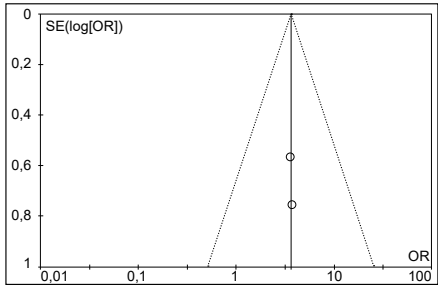


Figure 11. Funnel plot for the indicator “The incidence of elimination of HSV-1 and HSV-2 detected using PRC in patients with HHV infection”

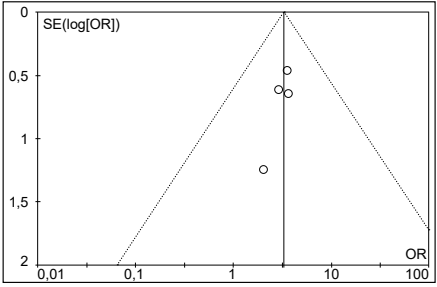


Figure 12. Funnel plot for the indicator “The incidence of positive effect of the treatment of inflammatory diseases of genitals caused by a mixed infection”

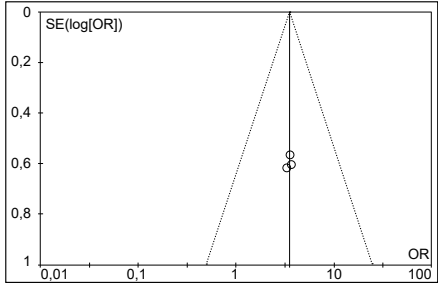


Figure 13. Funnel plot for the indicator “The incidence of improvement in the vaginal microflora condition in women with inflammatory diseases of the genitals caused by a mixed infection”

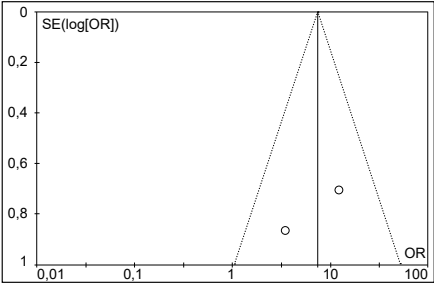


Figure 14. Funnel plot for the indicator “The incidence of positive dynamics of local changes in the cervix in women with inflammatory diseases of the genitals caused by a mixed infection”

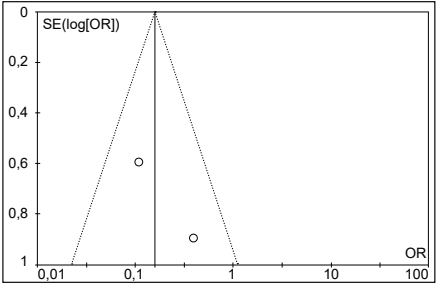


Figure 15. Funnel plot for the indicator “The incidence of ophthalmoherpes relapse”

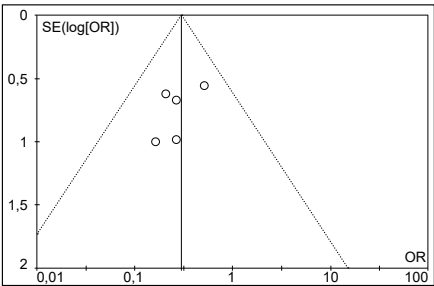


Figure 16. Funnel plot for the indicator “The incidence of genital herpes relapse”

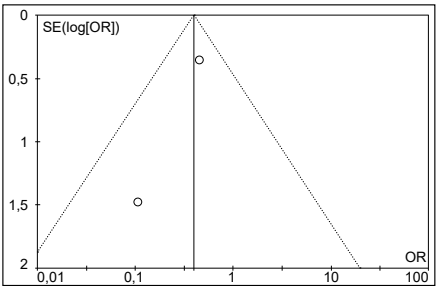


Figure 17. Funnel plot for the indicator “The incidence of relapse of chronic pyelonephritis caused by mixed infection”

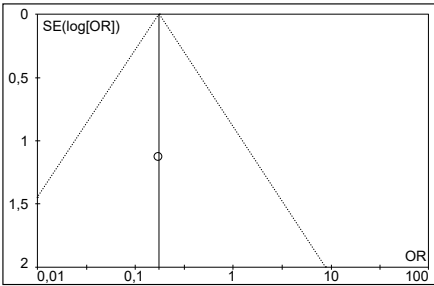


Figure 18. Funnel plot for the indicator “The incidence of relapse of inflammatory diseases of genitals caused by mixed infection”

Conclusions

Present meta-analysis confirms the efficacy of drug Proteflazid® in clinical practice in treatment of herpesvirus and mixed infections based on following results obtained.

1. Proteflazid® in patients over 18 y.o. with HHV infection (HSV-1, HSV-2) causes positive dynamics of clinical symptoms of diseases:

- the probability of an incidence of positive dynamics of clinical symptoms in patients with HHV infection after use of the drug Proteflazid® in the main group was 3.70 times higher than in the control group;
- the probability of an incidence of elimination of HSV-1 and HSV-2 detected by PCR in patients with HHV infection in the main group was 3.65 times higher than in the control group.

2. Proteflazid® in patients over 18 y.o. with mixed infection (HSV, bacteria, protozoa, fungi) causes positive dynamics of clinical symptoms of diseases:

- the probability of an incidence of positive effect of the treatment of inflammatory diseases of genitals caused by mixed infection in the main group was 3.28 times higher than in the control group;
- the probability of an incidence of improvement in vaginal microflora condition in women with inflammatory diseases of genitals caused by mixed infection in the main group was 3.55 times higher than in the control group;
- the probability of an incidence of positive dynamics of the local changes in the cervix in women with inflammatory diseases of genitals caused by mixed infection in the main group was 7.52 times higher than in the control group.

3. Proteflazid® in patients over 18 y.o. contributes to prevention of relapses of diseases after treatment completion:

- the probability of an incidence of relapse of ophthalmoherpes in the main group is 6.25 times lower than in the control group;
- the probability of an incidence of genital herpes relapse in the main group was 3.33 times lower than in the control group;
- the probability of an incidence of relapse of chronic pyelonephritis caused by infection in the main group was 2.5 times lower than in the control group;
- the probability of an incidence of relapse of inflammatory diseases of genitals caused by mixed infection in the main group was 5.88 times lower than in the control group.

Meta-analysis of results of clinical trials is indicative of the high efficiency of the drug Proteflazid® (drops) in the treatment of patients over 18 y.o. with herpesvirus (HSV-1, HSV-2) and mixed infections.

Proteflazid® in the therapy of various clinical forms of HHV infection promotes positive dynamics of main clinical symptoms of diseases, elimination of HSV-1 and HSV-2; prevention of diseases relapses (ophthalmoherpes, genital herpes) after treatment completion.

Therapy of mixed infections (HSV, bacteria, protozoa, fungi) using the drug Proteflazid® promotes positive dynamics of clinical implications of diseases, improvement in vaginal microflora condition and positive dynamics of local changes of the cervix in women with inflammatory diseases of genital organs as well as in prevention of relapses of genital inflammatory diseases and chronic pyelonephritis.

Taking into account the high clinical efficacy, and complex action on various links of virus infection development, the drug Proteflazid® can be recommended as an effective antiviral agent for the treatment of diseases caused by HHV infection (HSV-1, HSV-2) and mixed infections in adult patients.

References:

1. Kaminsky V. V., Litus O. I., Grynevych O. Y., et al.: PROTEFLAZID®: efficacy and safety in urogenital herpes virus infection (systematic review). Science and Education Studies, 2015; V3, 2 (16): 705–727.
2. Kaminsky V., Chernyshov V., Grynevych O., et al.: Proteflazid® and local immunity in diseases caused by human papillomavirus, herpesvirus and mixed urogenital infections. Polski Merkuriusz Lekarski, 2017; Tom XLII, 249: 51–56.
3. Kramarev S., Mikhailov V., Grynevych O., et al.: Meta-Analysis of clinical trials results of efficacy and safety of the drug Proteflazid® in the treatment and prevention of human herpesvirus infection in children. Asian Journal of Scientific and Educational Research, 2016; January-June 1 (19): 766–783.
4. Ventskivsky B., Grynevych O., Mikhailov V., et al.: Proteflazid® in the treatment and prevention of human herpesvirus infection in pregnant women: a meta-analysis of clinical trials results. American Journal of Science and Technologies, 2016; January-June, 1 (21): 983–1002.
5. Басв А. І., Алієва А. Т., Кузієва Г. Д. та ін.: Обґрунтування застосування Протефлазид® у комплексній терапії мікст – ППСІІ (1–4). Питання дерматології та венерології, 2011; 3–4 (47–48): 24–37.
6. Бенюк В. О., Гриневич О. Й., Усевич І. А. та ін.: Терапевтична ефективність супозиторіїв Протефлазид® при змішаній урогенітальній вірусно-бактеріальній інфекції. Здоров'я жінки, 2016; 2 (108): 162–166.
7. Бенюк В. О.: Порівняльна оцінка ефективності та переносимості препарату Протефлазид®, супозиторії виробництва ТОВ «Фармек Груп» та препарату Протефлазид®, краплі виробництва ПАТ «Фітофарм» у пацієнток з урогенітальною вірусно-бактеріальною інфекцією (2 фази). (Звіт). Національний медичний університет ім. О. О. Богомольця. К., 2015; 72.
8. Глей А. І.: Протефлазид® у лікуванні хворих на інфекційний мононуклеоз, спричинений вірусом Епштейна-Барр. Сучасні інфекції, 2005; 3–4: 121–124.
9. Гопчук О. М.: Корекція порушень менструального циклу у жінок з герпетичною інфекцією. Репродуктивне здоров'я жінки, 2006; 1: 187–190.
10. Гошко Е. Л., Рибалко С. Л., Матяш В. І. та ін.: Вивчення інтерферогенної та імуномодуючої активності Протефлазиду у хворих з герпесвірусним ураженням нервової системи. Лабораторна діагностика, 2005; 4 (34): 30–35.
11. Гринкевич Т. М.: Сучасні методи лікування генітального герпесу у жінок репродуктивного віку: Автореф. дис. к-та мед.наук: спец. 14.10.10 «Акушерство та гінекологія». К., 2005; 20.
12. Запольський М. Е.: Багатоморфна ексудативна еритема, асоційована з герпесвірусом. Епідеміологія та патогенетично обґрунтована терапія. Клінічна імунологія. Аллергологія. Інфектологія, 2012; 8 (57): 1–5.
13. Камілов Х. М., Йулдошева М. Н., Касимова М. С. та ін.: Оцінка ефективності препарату Протефлазид® у комплексному лікуванні герпетичних увеїтів. Офтальмологія, 2012; 2 (9): 62–64.
14. Камінський В. В., Шалько М. Н., Гриневич О. Й.: Протефлазид® у вагітних: системний огляд результатів післяреєстраційних спостережень за безпечністю та ефективністю застосування. Здоров'я жінки, 2014; 6 (92): 160–164.

15. Клименко П. М., Гурженко Ю. Н., Лукман І. Мухамад та ін.: Застосування препарату Протефлазід® для лікування хворих з урогенітальним герпесом. Здоров'я чоловіка, 2012; 4: 82–85.
16. Корнацька А. Г.: Порівняльна оцінка ефективності та переносимості препарату Протефлазід®, супозиторії виробництва ТОВ «Фармекс Груп» та препарату Протефлазід®, краплі виробництва ПАТ «Фітофарм» у пацієнток із загостренням герпетичної інфекції (2 фаза) (Звіт). ІПАГ НАНМ України, К. 2015; 83.
17. Корнацька А. Г., Ревенько О. Е., Гриневич О. Й. та ін.: Терапевтична ефективність та безпека супозиторіїв та крапель Протефлазід® у пацієнток із загостренням герпетичної інфекції. European Journal of Scientific Research, 2016; 1(13): 507–522.
18. Колесник М. О., Дріянська В. Є., Драннік Г. М. та ін.: Вплив імунореакції на стан цитокинової ланки І SLPI у хворих на пієлонефрит. Український журнал нефрології та діалізу, 2014; 1 (41): 19–29.
19. Лісовий В. Н., Яковлева Е. В.: Препарат Протефлазід® у терапії хворих на генітальний герпес. Здоров'я чоловіка, 2006; 1: 128–131.
20. Матяш В. І., Гриневич О. Й., Панасюк О. Л. та ін.: Протефлазід®: специфічна активність при інфекціях, спричинених вірусами герпесу в умовах доклінічного вивчення та ефективності/безпеки застосування у клінічній практиці (систематичний огляд). Yale Review of Education and Science, 2015; 1 (16): 423–462.
21. Нагорна В. Ф., Ніколаєва С. В.: Механізм імуноспрямованої дії Протефлазиду під час вагітності у пацієнток з невиношуванням вагітності герпесвірусної етіології. Здоров'я жінки, 2007; 1 (29): 111–114.
22. Нікіфорова Т. О., Пюрик В. Ф., Дикий О. Б. та ін.: Клініко-імунологічна ефективність Протефлазиду у хворих на Епштейна-Барр-вірусну інфекцію. Матеріали науково-практичної конференції і пленуму Асоціації інфекціоністів України, Тернопіль, 2004; 158.
23. Панасюк О. Л.: Етіопатогенетична терапія герпесвірусної інфекції із застосуванням Протефлазиду (Звіт). Інститут епідеміології та інфекційних хвороб ім. Л. В. Громашевського, К., 2007; 89.
24. Петруня А. М., Воротніков С. В.: Ефективність Протефлазиду у комплексній терапії хворих на рецидивуючий герпетичний кератит. Збірник наукових праць «Проблеми екологічної та медичної генетики та клінічної імунології», Київ – Луганськ – Харків; 2003: 309–314.
25. Рак Л. М., Юзько О. М.: Застосування індукторів ендогенних інтерферонів у комплексному лікуванні запальних захворювань придатків матки. Здоров'я жінки, 2013; 7 (83): 138–140.
26. Риков С. О., Знаменська М. А.: Сучасні погляди на етіологію та лікування герпетичних кератитів. Новини медицини та фармації, 2010; 324: 1–4.
27. Ромащенко О. В., Руденко А. В., Рибалко С. Л.: Клініко-мікробіологічна, імунологічна оцінка ефективності використання Протефлазиду у комплексній терапії запальних захворювань геніталій у жінок. Репродуктивне здоров'я жінки, 2005; 1: 89–93.
28. Рижко П. П., Рощенко Л. В.: Лікування герпесвірусної інфекції із застосуванням препарату Протефлазид у дерматовенерологічній практиці. Клінічна імунологія. Алергологія. Інфектологія Спецвипуск, 2012; 1: 1–3.
29. Сидоренко Є. В.: Фактори міжклітинної кооперації імунної системи та можливості імунореакції у хворих на хронічний пієлонефрит: Автореф. дис.к-та мед.наук: спец. 14.03.08 «Імунологія та алергологія». К., 2010; 24.
30. Скринь А. В.: Звіт про результати клінічних випробувань препарату Протефлазид® у терапії герпесвірусних інфекцій (Звіт). МДМСУ. М. 2008; 16.

31. Чоп'як В. В., Потьомкіна Г. О., Вальчук І. В.: Ефективність монотерапії препаратом Протефлазид® при лікуванні хворих на хронічну EBV-вірусну інфекцію в стадії реактивації. Імунологія та алергологія, 2008; 1: 3–9.
32. Шведюк С. В., Шеданія І. Е., Балкарова А. Л.: Протефлазид® у лікуванні уrogenітального хламідіозу, що поєднується з генітальним герпесом. Збірник наукових праць «Проблеми екологічної та медичної генетики та клінічної імунології», 2003, Київ – Луганськ – Харків; 376–380.

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4.2.THERAPEUTIC EFFICACY OF PROTEFLAZID® (SUPPOSITORY) AMONG THE PATIENTS WITH GENITOURINARY VIRAL-BACTERIAL INFECTION

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Abstract

The article reports of Phase II clinical trial results for PROTEFLAZID® (suppository), among the patients with genitourinary viral;bacterial infection. It had been shown high therapeutic efficacy PROTEFLAZID® (suppository) with comparable values for PROTEFLAZID® (drops in the form of vaginal tampon with the drug solution). Presented data allowed to draw conclusion that PROTEFLAZID® (suppository) can be recommended for implementation in clinical practice as a drug with high efficacy and good tolerability for the treatment of genitouri; nary viral;bacterial infection.

Key words: PROTEFLAZID® (suppository); PROTEFLAZID® (drops); *Deschampsia caespitosa* L., *Calamagrostis epigeios* L., flavonoids; viral-bacterial mixed infection, Herpes genitalis, Chlamydia.

Introduction

According to the WHO experts, 20 million new cases of herpes virus infection are estimated to occur each year in the developed countries [1, 5]. Chlamydia is also one of the most prevailing sexually transmitted diseases. There are no summary statistical data pertinent to the prevalence in Ukraine; however, 4 million new cases of chlamydia infections are recorded in the United States each year [2]. Chlamydia infection increases the risk of getting HIV and other sexually transmitted diseases (Siewert K. et al., 2005; Workowski K. A., Berman S. M., 2006).

Under these circumstances, the issue of developing new and improving existing methods of pathogenetic therapy in the treatment of the mixed urogenital viral-bacterial infections becomes especially relevant. There are reports of the resistant herpes infection and persistent chlamydia infection [3, 7]. Therefore, the rational etiotropic and immunorehabilitation therapy with the effect on the local immunity of the mucous membranes are the necessary stages allowing the reliable prevention of the disease chronicity and infection persistence. One of such new domestic products being in great demand in the specialists and population recently is the medicinal product PROTEFLAZID®.

The choice of the suppository dosage form is not accidental, as it allows the successful use of PROTEFLAZID® at the site of the infectious process in the treatment of patients of different ages and functional status.

The choice of the medicinal product for treatment was explained by the following characteristics of PROTEFLAZID®. The active substance of the medicinal product (flavonoids) inhibits the synthesis of RNA and DNA viruses in the infected cells due to the suppression of the virus-specific enzymes – RNA and DNA polymerases, thymidine kinase and reverse transcriptase – and exerts the immunotropic properties. It has been determined that the active substance facilitates the activation of the endogenous alpha- and gamma-interferon synthesis up to the physiologically active level (without refractory events) increasing the nonspecific resistance to the viral and bacterial infections. It was demonstrated in the clinical studies that the medicinal product PROTEFLAZID® (suppositories) restores the protective function of the vaginal and cervical mucosa by normalizing the local immunity factors (sIgA, lysozyme and complement component C₃). If used in the genital herpes, the medicinal product prevents the new rash elements, reduces the probability of dissemination and visceral complications and accelerates the healing of injuries. If used in the vaginosis, vaginitis and cervical inflammatory diseases, it promotes the restoration of the local immunity and more rapid and effective elimination of the pathogen. PROTEFLAZID® exerts the antioxidant activity, inhibits the free-radical processes preventing the accumulation of the lipid peroxidation products and enhancing the antioxidant status of cells. The medicinal product is the apoptosis modulator potentiating the action of the apoptosis-inducing factors, i.e.: by activating caspase-9 it facilitates the more rapid elimination of the virus-infected cells and primary prevention of the chronic diseases secondary to the latent viral infections.

Medicinal product PROTEFLAZID® (suppositories) received the approval during the clinical studies of tolerability (phase I). In addition, the safety and efficacy of the active substances of the medicinal product was proved in the process of the broad clinical use of PROTEFLAZID® (drops) since 2001.

The choice of the basic therapy product was based on Orders of the Ministry of Health of Ukraine No. 312 dated May 08, 2009 and No. 286 dated June 07, 2004 regulating the health care in the dermatovenerologic pathology. According to these orders, the treatment program used in the chlamydia infection involves the administration of azithromycin at the standard doses. The treatment programs used by our colleagues abroad include the recommendations to supplement the etiotropic therapy with the immuno-corrective products in the treatment of herpesvirus pathology and chlamydia infection [4, 8]

Study objective

Evaluation of efficacy of combination of medicinal product PROTEFLAZID® (suppositories) and Azimed, film coated tablets, 500 mg compared with the combination of medicinal product PROTEFLAZID® (drops) and Azimed, film coated tablets, 500 mg in the patients with the urogenital viral-bacterial infection.

Table 1. Disposition of subjects based on the gynecological history parameters

Parameter	Study group, n = 35		Control group, n = 35		Significance of differences*
	Abs.	%	Abs.	%	
Deliveries	10	28,6	14	40,0	0,4500
Abortions	15	42,9	12	34,3	0,6234
Miscarriage	9	25,7	7	20,0	0,7759
Menstrual irregularities	2	5,7	1	2,9	1,000

Note: * – evaluation was performed using Yates continuity-corrected chi-square test in combination with Fisher exact test.

Materials and methods

Study goals:

- investigate the therapeutic efficacy of the study drug PROTEFLAZID® (suppositories) in the patients with the urogenital viral-bacterial infection;
- investigate the therapeutic efficacy of the medicinal product PROTEFLAZID® (drops) in the patients with the urogenital viral-bacterial infection;
- compare the treatment outcomes in the study and control groups to evaluate the treatment efficacy.

Seventy (70) women aged 18 to 51 years were enrolled to the study; according to the randomization scheme they were assigned into two groups – the study and control groups (1:1). Patients from both groups received the basic therapy with the peroral Azimed, film coated tablets, 500 mg taken by the following dosage regimen: treatment day 1 – 1.0 g, treatment days 2–5 – 0.5 g per day.

Additionally, patients of the (study) group I were prescribed the investigational medicinal product PROTEFLAZID® (suppositories) for 14 days secondary to the basic therapy.

Patients of (control) group II were prescribed the reference product PROTEFLAZID® (drops) used as vaginal tampons with the product solution. To prepare the solution, 3.0 ml (72–75 drops) of the product were diluted in 20 ml of physiological saline (sodium chloride). Exposure time of the vaginal tampons was 30–40 minutes; the procedures had to be performed twice daily for 14 days.

Control of parameters was performed prior to the study start, after completion of the therapy course and 4 weeks after treatment completion. The conclusion on efficacy was made based on the evaluation of the results of test of vaginal/cervical mucosal epithelium smears for chlamydia DNA and HSV-1, HSV-2 DNA performed by PCR method prior to the completion of treatment course and follow-up. The medicinal product was considered to be effective if Chlamydia trachomatis DNA was absent in the material withdrawn from the affected area in the laboratory test performed twice by PCR method and if HSV DNA level was decreased by one or more points.

Study results and their discussion

The gynecological history data and eligibility to inclusion criteria were evaluated at the screening stage. See disposition of the patients based on the gynecological history parameters in Table 1.

The abortions, miscarriages and menstrual irregularities were prevailing in the gynecological history. No statistically significant differences in the examined groups were detected.

Seventy (70) women enrolled into the study received 10-day therapy course. After the treatment course was completed and 4 weeks after treatment course completion, the retest for chlamydia DNA and HSV DNA in the vaginal/cervical mucosal epithelium smears and HSV markers (IgG, IgM) was performed.

The proportions of subjects from each group with the Chlamydia trachomatis DNA detected in the vaginal/cervical mucosal epithelium smears in the laboratory test performed twice are provided in Figure 1.

Chlamydia DNA was detected at screening in 100% of patients from each group as it was inclusion criteria in the study. After 14-day treatment course was completed, chlamydia DNA was not detected in any patient from each group.

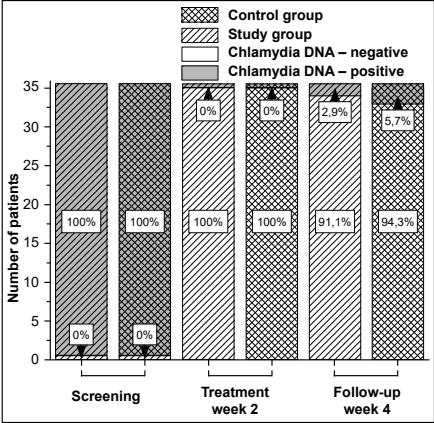


Figure 1. Detection of chlamydia DNA over time

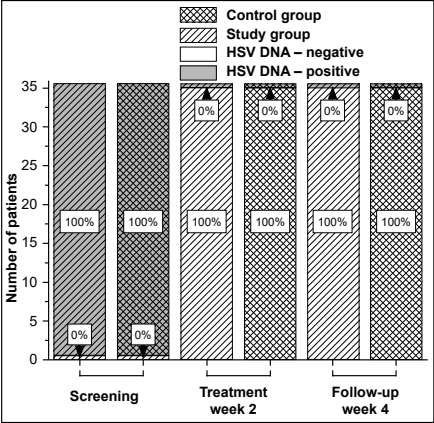


Figure 2. Detection of HSV DNA over time

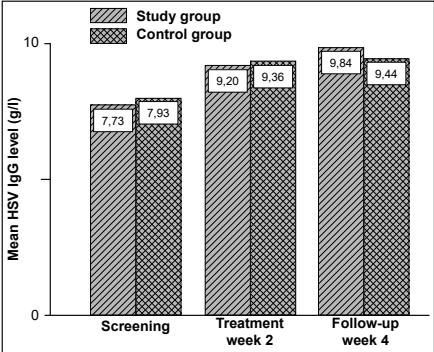


Figure 3. Changes of IgG parameter (g/l) over time

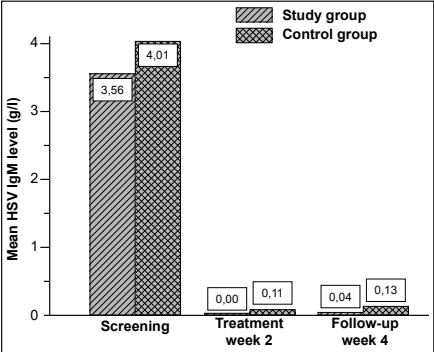


Figure 4. Changes of IgM parameter (g/l) over time

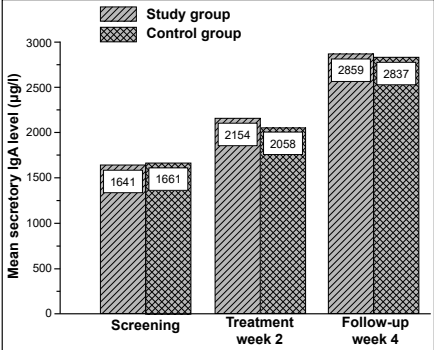


Figure 5. Changes of secretory IgM parameter (µg/l) over time

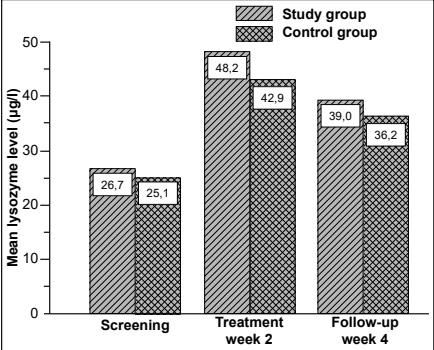


Figure 6. Changes of lysozyme parameter (µg/l) over time

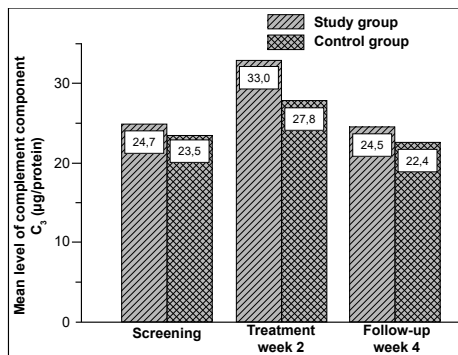


Figure 7. Changes of complement component C₃ parameter (µg/protein) over time

HSV DNA was detected in the pre-treatment cervical mucosal epithelium smears in all subjects from both groups (it also corresponds to the inclusion criteria).

HSV DNA was not detected in any subject prior to the treatment course completion and after 4-week follow-up completion (Figure 2).

Based on the provided data, the conclusion was made about the significantly decreased level of DNA markers of Chlamydia trachomatis and HSV in both groups.

Results of evaluation of HSV IgG, IgM changes over the time in groups are provided in Figure 3 and Figure 4.

The presented data allow indicating the significantly decreased levels of HSV IgM serological markers and increased levels of HSV IgG serological markers in both groups.

After the treatment course completion and 4 weeks after the treatment course completion, the retest for local immunity parameters was performed (secretory IgA, lysozyme, complement component C₃). The results of evaluation of local immunity parameters over time are provided in Figures 5–7.

The significantly increased secretory IgA and lysozyme levels were observed in both groups on Week 2 and Week 4 compared with the screening period. The complement component C₃ level was significantly increased prior to the treatment course completion in the study group and returned to the baseline level prior to the follow-up completion. There were no significant changes of the complement component C₃ level in the control group.

Conclusion on the efficacy of the medicinal product PROTEFLAZID® (suppositories) compared with the medicinal product PROTEFLAZID® (drops) used as vaginal tampons with the product solution in the patients with the urogenital viral-bacterial infection was made according to the approach based on the confidence intervals. The calculation results are provided in Table 2.

Based on the provided data, the treatment was considered to be effective in 34 (97.1%) patients from the study group and in 33 (94.3%) patients from the control group.

No serious side effects or events were recorded in the patients during the study. None of the patients discontinued the study early due to the adverse event.

Table 2. Bounds of 95% confidence interval for the difference between the proportions with positive results

Statistical parameter	Value
Type I error probability, α	0.025
Percentage point of standardized normal distribution, α	1.96
Efficacy zone, δ (%)	=20%
Proportion of positive consequences for study group, %	97.1
Size of study group	35
Proportion of positive consequences for control group, %	94.3
Size of control group	35
Difference of proportions, %	2.8
Standard error of difference	5.523
Lower bound of 95% CI	=16.53
Upper bound of 95% CI	5.13

The compared medicinal products had no negative effects on the blood pressure, heart rate and body temperature: after the clinical study was completed, the patients from both groups did not demonstrate the negative changes of those parameters compared with the pre-treatment baseline levels. There were no negative changes of the laboratory parameters in any patient. There were no cases of exacerbation of the existing chronic diseases; no anaphylactic reactions, delayed reactions or critical variations of the hemodynamic and basic laboratory parameters were recorded. Based on the provided data, the evaluation of tolerability of the study drugs was performed (Table 3).

Table 3. Evaluation of tolerability of the study drugs

Tolerability	Study group, n = 35		Control group, n = 35	
	Rate	Proportion, %	Rate	Proportion, %
Good	35	100, 0	35	100, 0
Satisfactory	0	0	0	0
Unsatisfactory	0	0	0	0

Special attention should be paid to certain aspects of this study. In particular, chlamydia DNA was detected at screening in 100% of subjects from each group as it was the inclusion criteria in the study. After 14-day treatment course was completed, chlamydia DNA was not detected in any patient from each group. Chlamydia DNA redetected at the last examination after 4-week follow-up (in 1 patient from the study group and in 2 patients from the control group) may indicate re-infection.

Changes of the local immunity parameters observed in the groups over the time involved the positive changes of the secretory IgA, lysozyme and complement component C₃ levels indicating the positive effect of the treatment on the local immunity condition. Similar changes were observed in the studies of other authors [6]. However, the specified changes were less marked in the control group over the time. Probably this fact indicates the more significant effect of the medicinal product PROTEFLAZID® (suppositories) on the local immunity condition compared with the drops.

Conclusions

1. The study drug PROTEFLAZID® (suppositories) is a highly effective product in the patients with the urogenital viral-bacterial infection of mixed etiology (herpes simplex viruses and chlamydia). Based on the study protocol criteria, treatment efficacy was 97.1%.

2. Based on the data obtained during the study, the conclusion was made that the efficacy of the study drug PROTEFLAZID® (suppositories) is not inferior to one of medicinal product PROTEFLAZID® (drops) used topically as vaginal tampons with the product solution.

3. Significantly decreased HSV DNA load was observed in both group compared with the baseline level (it was detected in all patients at the screening stage). After 14-day treatment course completion and after 4-week follow-up completion, no HSV DNA was detected in the vaginal/cervical mucosal epithelium smears in any patient.

4. Significantly decreased (compared with the baseline) rate of detected chlamydia DNA was observed in both groups. After 14-day treatment course was completed, chlamydia DNA was not detected in any patient; after 4-week follow-up was completed, chlamydia DNA was detected in 1 patient from the study group and in 2 patients from the control group.

5. Additional evidence of the therapy efficacy in both groups is the significantly increased parameters of the local immunity. In particular, secretory IgA and lysozyme levels were increased already by treatment day 14 remaining significantly high during the follow-up period (secretory IgA: 1 641.9 µg/l – at the screening stage; 2 154.2 µg/l – on day 14; 2 859.3 µg/l – prior to 4-week follow-up completion; lysozyme: 26.7 µg/l – at the screening stage; 48.2 µg/l – on day 14; 39.0 µg/l – prior to 4-week follow-up completion); complement component C₃ level was increased in the study group by treatment day 14 and returned to the baseline level prior to 4-week follow-up completion (24.7 µg/protein – at the screening stage; 33.0 µg/protein – on day 14; 24 µg/protein – prior to 4-week follow-up completion for complement component C₃ levels).

6. HSV IgM serological markers were decreased in both groups after completion of 14-day treatment course from 3.56 to 0.00 and after completion of 4-week follow-up – 0.11 in the study group and from 4.01 to 0.01 and 0.13, respectively in the control group.

7. Based on the provided data, it is possible to recommend the medicinal product PROTEFLAZID® (suppositories) as the efficacious and safe antiviral medication for the treatment of patients with the mixed urogenital viral-bacterial infection.

References:

1. Adler M. W. Sexually transmitted diseases control in developing countries // *Genitourin. Med.* – 1996. – V. 72 (2). – 83–88.
2. Alexander L. L., Ireiman K., Clarc P. A national survey practitioner chlamydia knowledge and treatment practices of female patients // *Nurse Practitioner.* – 1996. – V. 21 (5), №48. – P. 51–54.
3. Bacon T. M., Levin M. J., Leary J. J., et al. Herpes simplex virus resist ance to acyclovir and penciclovir after 2 decades of antiviral therapy // *Clinical Microbiology Reviews.* – 2003. – V. 16, №1. – P. 114–128.
4. Leung D. T., Sacks S. L. Current Recommendations for the treatment of genital herpes // *Drugs.* – 2000. – V. 60 (6). – P. 1329–1352.

5. Looker K. J., Garnett G. P. A systematic review of the epidemiology and interaction of herpes simplex virus types 1 and 2 // *Sexually Transmitted Infections*. – 2005. – V. 81. – P. 103–107.
6. Глазкова Л. К., Герасимова Н. М. Стан факторів неспецифічного захисту організму жінок при хламідіозі // *Вісник дерматології та венерології*. – 1998. – №1. – З. 7.
7. Гомберг М. А., Соловійов А. М., Черноус А. Д. Обґрунтування імунотерапії при лікуванні рецидивуючого уrogenітального хламідіозу // *ІПСШ 2000*. – №2. – С. 30–35.
8. Рекомендації з лікування герпесу щодо статевих органів (адаптовано з Guidelines for Management of Genital Herpes в Новій Зеландії. 7th Ed. – 2004) // *Здоров'я жінки*. – 2006. – №3 (27). – С. 167–172.

Reference: V. O. Benyuk, O. Y. Hrynevych, I. A. Usevych, I. S. Sikachova, M. V. Shepetko. *Therapeutic efficacy of PROTEFLAZID® (suppository) among the patients with genitourinary viral-bacterial infection* // *Womens Health*. – 2016. – No.2 (108). – pp. 162–166.

4.3. META-ANALYSIS OF CLINICAL STUDIES ON THE EFFICACY OF FLAVONOIDS IN VIRAL AND VIRAL-BACTERIAL DISEASES IN CHILDREN

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Abstract

In the work shows a metaanalysis of clinical studies of the effect of flavonoids on the effectiveness of reducing fever and intoxication syndrome during viral and viral-bacterial diseases in children. A meta-analysis involving 2,699 children, confirms the efficacy and safety of drugs that contain flavonoids: PROTEFLAZID®, FLAVOVIR®, Flavozid® (Ukraine) and proves an anti activity of these drugs.

Key words: PROTEFLAZID®, FLAVOVIR®, Flavozid®, meta-analysis, viral diseases, children, intoxication syndrome, fever.

Introduction

Viral and viral-bacterial diseases are the most common and most relevant pathology of the childhood holding a leading place in the structure of childhood diseases. The diseases are characterized by the multi-organ tropism; they cause damage of almost all organs and systems of the child's body: respiratory, gastrointestinal, cardiovascular, nervous systems, etc. Moreover, in children, compared to adults, the more severe disease is observed, with the frequent serious complications and chronicity of the pathology.

Treatment of the viral and viral-bacterial diseases should be a combination, etiopathogenetic therapy, contribute to the prevention of recurrences and complications, be effective, safe and take into consideration the characteristic features of the child's body.

The medicinal products with the combined effect simultaneously possessing the antiviral and immuno-corrective activity, proved efficacy and safety involve the medications containing flavonoids of *Herba Deschampsia caespitosa* L. and *Herba Calamagrostis epigeios* L. wild grasses: PROTEFLAZID® and FLAVOVIR® (SMC "Ecopharm", Kyiv, UKRAINE).

The preclinical studies conducted in the different research institutes and clinics proved that the medicinal products containing flavonoids possess the direct antiviral, immunotropic, apoptosis-modulating and antioxidant effects. Such pharmacodynamics of the medicinal products makes their use appropriate in the treatment of the acute, latent and chronic forms of the viral and viral-bacterial infections.

Objective

To confirm that the flavonoids effectively relieve the intoxication syndrome and fever in the viral and viral-bacterial diseases in children and form the evidence base, perform the meta-analysis of the clinical studies and long-term results of using medicinal products PROTEFLAZID®, FLAVOVIR® and Flavozid® by the following parameters: children without age limit, infectious diseases of viral and viral-bacterial etiology, presence of the intoxication syndrome and fever.

Table 1. Identifier of publications, nosology, number of children included in the meta-analysis according to the results of the study

No.	Identifier	Nosology	Number of children
1	Kriuchko T. O., 2002	Neuroinfection	20
2	Shapovalova I. A., 2003	Tonsillitis	220
3	Nikiforova T. A., 2004	Epstein-Barr viral infection	26
4	Hlei A. I., 2005	Infectious mononucleosis	27
5	Usacheva Ye. V., 2005	Infectious mononucleosis	38
6	Chernyshova Ye. Ye., 2005	Herpes virus infection	199
7	Herasimov S. V., 2006	Herpetic gingivostomatitis	38
8	Kriuchko T. O., 2006	Herpes virus infection	62
9	Herasimov S. V., 2007	Herpetic gingivostomatitis	36
10	Yulish Ye. I., 2007	Recurrent obstructive bronchitis	105
11	Kharchenko Yu. P., 2007	Infectious mononucleosis	60
12	Kramarev S. O., 2007	Herpes virus infection	80
13	Kramarev S. O., 2007	Epstein-Barr viral infection	35
14	Kriuchko T. O., 2008	Herpes virus infection	58
15	Kramarev S. O., 2008	Epstein-Barr viral infection	35
16	Prokhorova M. P., 2008	ARVI	35
17	Yulish Ye. I., 2008	Herpes virus infection	350
18	Volosovets O. P., 2008	ARVI	35
19	Yulish Ye. I., 2009	ARVI	80
20	Sinchenko P. I., 2009	ARVI	120
21	Turlibekov S. S., 2011	Cytomegalovirus infection	48
22	Shamsiev F. M., 2011	Acute pneumonia	169
23	Yulish Ye. I., 2011	Herpes virus infection	130
24	Kramarev S. O., 2011	Epstein-Barr viral infection	60
25	Yulish Ye. I., 2011	Herpes virus infection	130
26	Zalizuiuk A. A., 2011	Community-acquired pneumonia	120
27	Kramarev S. O., 2012	Herpes virus infection	80
28	Tokarchuk N. I., 2012	ARVI and influenza	50
29	Yulish Ye. I., 2013	Herpes virus infection	140
30	Kramarev S. O., 2014	Infectious mononucleosis	243
Total			2699

Study materials and methods

The meta-analysis included 30 clinical studies involved 2 699 children aged 3 to 18 years for the period from 2002 to 2014. We selected 30 publications with the results of the cohort clinical studies meeting the search criteria. During the initial selection, not all studies met the standard requirements of meta-analysis due to the lack of the reference group; some studies were presented as the cohort trials without the reference group. Therefore, to evaluate the efficacy of the medicinal products containing flavonoids in children with the fever and intoxication syndrome, only the studies with the reference group were included into the meta-analysis. Based on the selected studies, the identifier of publications was created including the main author, year of publication, nosology and number of children receiving the medicinal products containing flavonoids (Table 1). Based on the evaluations of the efficacy of flavonoids effect on the body temperature and intoxication syndrome in children with the viral and viral-bacterial diseases obtained in some studies, we performed the summarization and quantitative evaluation of the clinical effects in the form of the meta-analysis with an assessment of the statistical significance of the results. To perform the statistical analysis, we used the licensed version of Stat 12 program.

Study results and their discussion

We selected five studies meeting the analysis criteria to evaluate the rate of body temperature normalization during the follow-up period. Those studies involved children with the following pathologies: Epstein-Barr viral infection (EBV infection), infectious mononucleosis and herpes virus infection.

Considering the different nosologies and severity of the disease, the important aspect involved the body temperature normalization at the control points of the follow-up. The study recorded the gradually decreased fever without the significant change of trends over the time. The statistically significant differences of the fever normalization at the control points of the follow-up between the study and control groups were not detected in all studies.

The study conducted by Nikiforova T. A. et al. (2004) demonstrated the tendency towards higher rate of temperature normalization secondary to the administration of flavonoids versus control group – odds ratio (OR) = 3.5 (95% CI: 0.7–17.8), $p = 0.126$ [14].

In the study conducted by Kramarev S. O. et al. in 2012 in the patients with in the herpes virus infection, it was possible to compare the results in the groups: OR = 1 [10].

The study conducted by Kramarev S. O. et al. in 2014 in the patients with in the infectious mononucleosis demonstrated the statistically significant differences and more favorable results in the study group: OR = 10.4 (95% CI: 1.2–84.4), $p = 0.012$ [8]. Evaluation of the model heterogeneity: $I^2 = 0.0\%$, $p = 0.430$ indicating the homogeneity of effects of the increased rate of fever relief in the different studies. The integral assessment of the odds ratios indicates the statistically significant increase of the probability of the temperature normalization in children secondary to the administration of medicinal products containing flavonoids by 4.64 times compared with the control group receiving the basic therapy: OR = 4.64 (95% CI: 1.5–14.3). The obtained heterogeneous results indicate the sensitivity of the rate of body temperature normalization to the parameters of severity and nosology of the disease, but no dependence on the dosage and dosage form of the medicinal product was revealed (Figure 1).

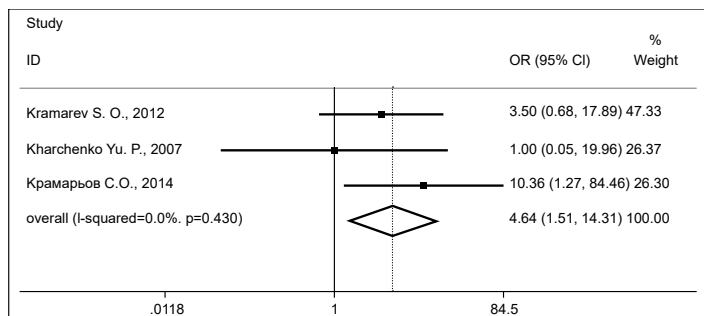


Figure 1. Meta-analysis of probability of fever elimination at the end of the study or at the control points of follow-up (evaluation of odds ratio in children with the viral diseases)

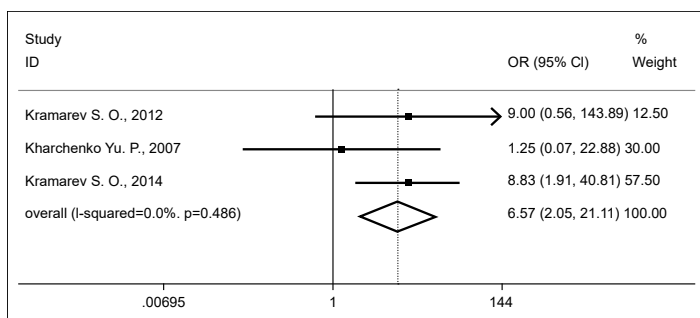


Figure 2. Meta-analysis of probability of intoxication syndrome elimination (evaluation of odds ratio [OR] in children with the viral diseases)

Based on the results of the expert evaluation of the primary results of the studies by the criterion of rate of intoxication syndrome at the control points of the follow-up, we selected five studies: two cohort trials and three comparative trials. Meta-analysis involved only comparative trials indicating the significantly increased probability of intoxication syndrome elimination in the children receiving the study drugs compared with the control group: Kramarev S. O. et al. (2012): OR = 9.0 (95% CI: 0.6–143.9), $p > 0.05$ [10]; Kramarev S. O. et al., (2014): OR = 8.8 (95% CI: 1.9–40.8), $p < 0.01$ [8]; Kharchenko Yu. P. et al. (2007): OR = 1.25 (95% CI: 0.1–22.9), $p > 0.05$ [25]. The integral assessment indicates the significantly increased (odds ratio [OR] = 6.6 (95% CI: 2.0–21.1), $p < 0.01$) therapy efficacy manifesting in the relief of intoxication syndrome in the children receiving flavonoids. Evaluation of the model heterogeneity: $I^2 = 0.0\%$, $p = 0.486$ indicating the homogeneity of effects of the increased rate of intoxication syndrome relief in the different studies (Figure 2). Cohort clinical studies without reference group confirm the efficacy of flavonoids in the relief of the intoxication syndrome in children: Kramarev S. O. et al. (2008) noted that normalization after 14 days was recorded in 55.0% of patients with the chronic EBV infection [11]; Kramarev S. O. et al. (2014) – normalization on day 14 was recorded in 73.3% of patients with the infectious mononucleosis [8]. Kriuchko T. A. et al. (2006) recorded the improved

overall health status in more than third of patients due to the decreased intensity of intoxication symptoms already on the fifth day of treatment with the flavonoids [12]. Sinchenko P. I. et al. (2009) stated about the decreased intoxication in 90% of sick children already on treatment day 2–3 secondary to the therapy with flavonoids [21]. In the study conducted by Zaliziuk A. A. (2011) [7], the reduced intoxication syndrome (6.67 ± 0.32 days versus 10.7 ± 0.8 days in reference group, $p < 0.05$) was observed in the group of children who received the flavonoids (Figure 2).

Information about the comparative analysis of the duration of fever and intoxication syndrome in children secondary to the administration of the study drugs is significantly more common. Meta-analysis of fever duration included 10 studies of the following nosologies: influenza, ARVI, tonsillitis, infectious mononucleosis, cytomegalovirus and herpesvirus infections. All below mentioned studies demonstrated the reduced duration of fever secondary to the administration of the products containing flavonoids compared with the control group (basic therapy). Mean time of fever duration reduction was 3.1 days with the significant variations in the individual studies: Kramarev S. O. et al. (2012): $\Delta = -1.4$ days [10], Herasimov S. V. et al. (2006): $\Delta = -5$ days [6]; Turlibekov S. S. (2011): $\Delta = -2.5$ days [24]; Herasimov S. V. (2007): $\Delta = -2$ days [2]; Kharchenko Yu. P. et al. (2007): $\Delta = -5$ days [24]; Yulish Ye. I. (2008): $\Delta = -1.29$ days [19]; Yulish Ye. I. et al. (2009): $\Delta = -6.4$ days [15]; Tokarchuk N. I. et al. (2012): $\Delta = -4.6$ days [23]; Shapovalova I. A. (2002): $2\Delta = -1.3$ days [27]; Kramarev S. O. et al. (2014): $\Delta = -1.4$ days [8]. Evaluation of the model heterogeneity: $I^2 = 93.5\%$, $p = 0.0001$ indicating the heterogeneity of effects of the reduced fever duration in the different studies.

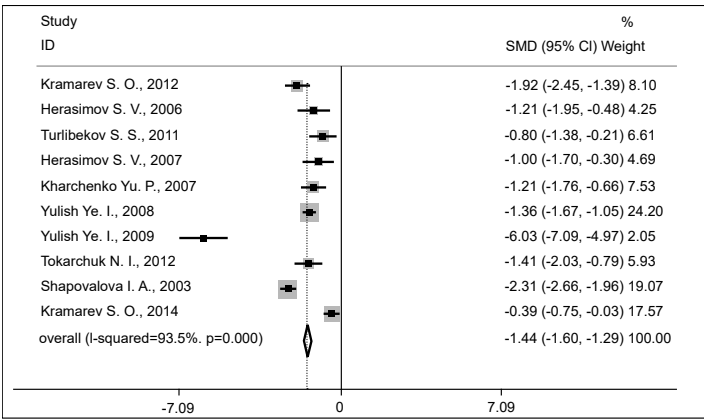


Figure 3. Meta-analysis of fever duration in the children from study group (PROTEFLAZID®, FLAVOVIR® and Flavozid®) compared with the control group (standardized mean difference for children with the viral and viral-bacterial diseases)

“Effect size” parameter was the basis for the analysis of change of the quantitative variables and their comparative evaluation. In literature this parameter is referred to as the standardized mean difference (SMD). The standardized mean difference is used as the effective statistics in the meta-analysis when the studies evaluate the outcome but the

measurements are presented in the different units or have different absolute size of changes and variability of parameters. In this case, it is necessary to standardize the study outcomes using the single scale before they can be combined. The standardized mean difference reflects the effect size obtained as a result of the intervention (administration of medicinal product) in each study taking into consideration the variability of the outcomes when compared with the control group, i.e. the evaluation of the most significant reduction of the fever duration in the study group (Figure 3).

The integral size of the standardized difference by the fever duration is $SMD = -1.4$ (95% CI: -1.6 to -1.3), $p < 0.001$. According to the Cochrane guidelines (Paul D Ellis / The Essential Guide to EFFECT SIZES, – UK, Cambridge, 2010. – P. 41), if the absolute value of the standardized difference (effect size) is greater than 0.8, it is generally considered a large effect size – significantly exceeded efficacy in the study group with the reduced fever duration compared with the control group. The significant heterogeneity of outcomes (heterogeneity factor $I^2 = 93.5\%$) may be explained by the different study parameters (severity of the pathological process, different diagnoses, etc., Figure 3).

In addition to the fever duration, intoxication syndrome duration is also of the significant clinical importance. Using that parameter, we selected six cohort comparative studies involved the following nosologies: influenza, ARVI, community-acquired pneumonia, infectious mononucleosis, cytomegalovirus and herpesvirus infections. Mean time of intoxication syndrome duration reduction was 3.1 days compared with the control group with the significant variations in the individual studies: Kramarev S. O. et al. (2012): $\Delta = -3.3$ days [10], Yulish Ye. I. et al. (2009): $\Delta = -6.4$ days [15]. Evaluation of the model heterogeneity: $I^2 = 96.0\%$, $p = 0.0001$ indicating the heterogeneity of effects of the reduced duration of intoxication syndrome in the different studies.

The standardized effect size of the reduced duration of intoxication syndrome in the study group secondary to the administration of the study drugs is $SMD = -1.84$ (95% CI: -2.1 to -1.6), $p = 0.0001$. The value of the more marked effect of the reduced duration of intoxication syndrome in the study group is statistically significant in all presented studies ($p < 0.05$) (Figure 4).

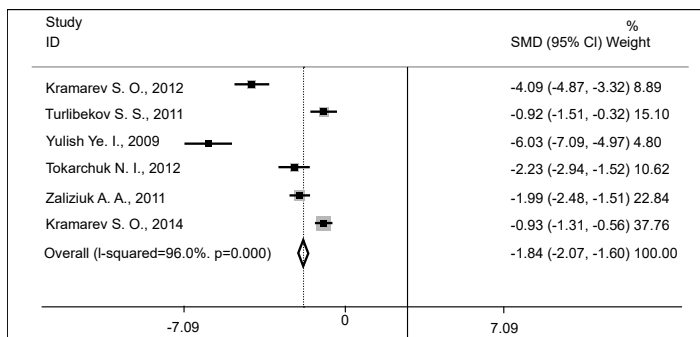


Figure 4. Meta-analysis of duration of intoxication syndrome in the children from study group (PROTEFLAZID®, FLAVOVIR® and Flavozid®) compared with the control group (standardized mean difference for children with the viral and viral-bacterial diseases)

Laboratory criteria for the fever and intoxication manifestations are the changes of the hemogram parameters – leukocytosis and lymphopenia. Normalization of these parameters can serve as the assessment of the superior efficacy of flavonoids. Previously performed analysis allowed selecting three studies in which it was possible to compare the rate of normalization of leukocyte and lymphocyte levels in the infectious mononucleosis (Usacheva Ye. V. et al., 2005 [4]), cytomegalovirus infection (Turlibekov S. S., 2011 p. [24]) and EBV infection (Nikiforova T. O. et al., 2004 [14]). At the end of the study, the high level of normalization of the above mentioned parameters was observed in the study group: Nikiforova T. O. et al. (2004): 92.9% of patients [14], Turlibekov S. S. (2011): 91.3% [24], Usacheva Ye. V. et al. (2005): 88.2% [4]. In the reference group, restoration of the normal levels of WBC and lymphocytes was recorded much less frequently (58.3%, 72.0%, 52.4%, respectively). Evaluation of the model heterogeneity: $I^2 = 6.40\%$, $p = 0.343$ indicating the homogeneity of effects of the increased rate of normalized WBC and lymphocyte levels in the different studies (Figure 5)

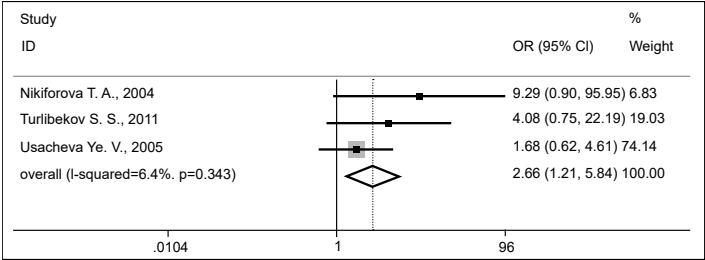


Figure 5. Meta-analysis of the rate of normalization of WBC and lymphocyte counts (assessment of the odds ratios [OR]) in the children with herpesvirus infections

The obtained results indicate the significantly increased treatment efficacy if flavonoids are added to the therapy regimen. At the end of the study it is manifested by the increase of the probability of normalization of the WBC and lymphocyte levels in the study group by 2.7 times compared with the control group (odds ratios [OR] = 2.66 (95% CI: 1.2–5.8), $p = 0.015$) (Figure 5).

When the flavonoids are added to the therapy regimen of the viral and viral-bacterial diseases, not only the increased clinical efficacy of the treatment is important. The relevant aspects involve the long-term outcomes of treatment and recurrence rate. To evaluate the recurrence rate, the long-term follow-up period is needed; it is more common in the cohort clinical studies without the reference group. The performed analysis of the long-term outcomes of treatment in general confirms the superior efficacy of treatment regimens with the added flavonoids (PROTEFLAZID®, FLAVOVIR® and Flavozid®). In the study conducted by Hlei A. I., 2005 ($n = 27$), no recurrences were recorded in the 6-month follow-up period [3]. In the study conducted by Chernyshova Ye. Ye., 2005 ($n = 199$), the relief of the acute events of infection was recorded in 67% of children, the transition of the recurrent chronic herpesvirus infection into latent one was observed in 64% of children [26]. In all followed-up children, the number and duration of the recurrent respiratory diseases and the rate of the complications decreased by 2.5 times [26]. Yulish Ye. I. et al., 2007 ($n = 199$) wrote that the intoxication events usually disappeared on the first or second treatment day in the children with the herpesvirus infection secondary to administration of medicinal products containing flavonoids [17]. In children with the chlamydial and mycoplasmal pneumonia, signs of intoxication were resolved mainly on the second day of treatment; during six months,

the complete absence of the obstructive bronchitis recurrences was recorded in half of the children from the group with the active infection and in 2/3 of the patients from the second group with the latent infection. Flavonoids used in the combination therapy of children contribute to the elimination of the acute signs of infection in 67% of patients and disease transition from the recurrent into latent one in 64% [17]. Kriuchko T. O. et al., 2008 (n = 58) recommend using flavonoids as the anti-relapse treatment of herpes virus infection; the follow-up results demonstrate that 17% of patients did not have diseases recurrences, 27.8% of patients did not have recurrences during the first year and had 1–2 recurrences during the second year, 41.4% of patients had once recurrence episode per year [13]. The study conducted by Kramarev S. O., 2008 (n = 35) demonstrated the significant decrease of the following clinical symptoms: intoxication, CNS dysfunction, impaired appetite, fever, nasopharyngeal lesions ($p < 0.05$) three months after the treatment was commenced [1]. Data provided by Volosovets O. P., 2007 (n = 35) indicate the high treatment efficacy in 30 (85.7%) patients and moderate efficacy in 5 (14.3%) patients with the herpesvirus infection [20]. After 3-month therapy in the herpesvirus infected frequently and chronically ill children (FCIC), Yulish Ye. I. et al., 2011, 2013 (n = 243) recorded the significant (almost by 1.5 times) decrease of the ARVI incidence within a year and 1.4-fold decrease of the complication rate; follow-up (up to 12 months) of the FCIC who received the flavonoids showed the decreased ARVI incidence by almost 3 times, reduced duration of the disease episode – by 1.5 times and complication rate – by 35% together with the normalization of the immunity parameters [28, 29]. In the study conducted by Kramarev S. O. et al. in 2014, number and duration of the recurrent respiratory diseases and their complication rate decreased in children after they received treatment with flavonoids [8].

No side effects were recorded in the above mentioned studies while using the medicinal products. Also, no events leading to flavonoids dose reduction were observed in the clinical use of the medicinal products.

Conclusions

1. Meta-analysis of 30 clinical studies involved 2 699 children with the viral and viral-bacterial diseases with the hyperthermic and intoxication syndromes indicates the efficacy and safety of using the flavonoids (medicinal products PROTEFLAZID®, FLAVOVIR® and Flavozid®) in children.

2. The comparative analysis proved the superior efficacy of using the above mentioned medicinal products by all studied characteristics – relief of fever, intoxication syndrome, normalization of WBC and lymphocyte levels, reduction of recurrence rate.

3. No side effects were observed.

4. No fever-related symptoms were recorded secondary to the administration of the medicinal products containing flavonoids.

5. There were no temperature reactions that required dosing adjustment.

6. No clinically significant laboratory abnormalities were recorded secondary to using therapy regimens with the study drugs.

References:

1. Використання препарату «Флавозід» у комплексному лікуванні хронічної активної Епштейна-Барр вірусної інфекції у дітей / Крамарьов С. О., Виговська О. В., Палатна Л. О. [та ін.] // Суч. педіатрія. – 2008. – Т. 20, №3. – С. 111–114.

2. Герасимов С. В. Етіотропне лікування Флавозідом гострого герпетичного гінгівостоматиту у немовлят і дітей раннього віку / С. В. Герасимов // Перинатол. та педіатрія. – 2007. – Т. 30, №2. – С. 75–78.
3. Глей А. І. Протефлазід у лікуванні хворих на інфекційний мононуклеоз, спричинений вірусом Епштейна-Барр / А. І. Глей // Суч. інфекції. – 2005. – №3/4. – С. 121–124.
4. Досвід використання препарату Протефлазід при інфекційному мононуклеозі у дітей / О. В. Усачова, О. М. Фірюліна, О. В. Анікіна, Самойленко // Репродуктивне здоров'я жінки. – 2005. – №4. – С. 192–194.
5. Ефективність препарату Флавозід у дітей з ГРВІ, ускладненою бронхообструктивним синдромом / М. П. Прохорова, О. П. Волосовець, С. П. Кривопустов, Н. Г. Бічкова // Експеримент. і клін. медицини. – 2008. – №4. – С. 129.
6. Ефективність Протефлазиду при первинному герпетичному гінгівостоматиті у дітей раннього віку / С. В. Герасимов, В. Н. Чирун, Н. В. Вітківська [та ін.] // Репродуктивне здоров'я жінки. – 2006 – Т. 26. – №2. – С. 142–144.
7. Залізюк О. О. Особливості сучасного перебігу негоспітальних пневмоній та їх лікування у дітей раннього віку: автореф. дис. ... спец. 14.01.10 «Педіатрія» / О. О. Залізюк. – Х., 2011. – 20 с.
8. Крамарьов С. О. Лікування інфекційного мононуклеозу у дітей / С. О. Крамарьов // Суч. педіатрія. – 2014. – Т. 59, №3. – С. 1–8.
9. Крамарьов С. О. Досвід використання препарату Флавозід при Епштейна-Барр вірусною інфекцією у дітей / С. О. Крамарьов, О. В. Виговська // Суч. педіатрія. – 2011. – Т. 39, №5. – С. 1–6.
10. Крамарьов С. О. Звіт про проведення клінічного дослідження ефективності та переносимості препарату Флавозід у дітей з герпесвірусними інфекціями / С. О. Крамарьов, О. В. Виговська // Клін. імунол. Аллергол. Інфектол. – 2012. – Т. 53, №4. – С. 1–8.
11. Крамарьов С. О. Хронічні форми Епштейна-Барр вірусної інфекції ції у дітей: сучасні підходи до діагностики та лікування / С. О. Крамарьов, О. В. Виговська // Суч. педіатрія. – 2008. – Т. 19, №2. – С. 103–108.
12. Крючко Т. О. Актуальні питання лікування герпетичної інфекції у дітей / Т. О. Крючко, Ю. М. Кінаш // Перинатол. та педіатрія. – 2006. – Т. 27. – №3. – С. 60–63.
13. Крючко Т. О. До питань профілактики поствірусного синдрому втомлюваності у дітей з герпетичною інфекцією / Т. О. Крючко, Ю. М. Кінаш // Перинатол. та педіатрія. – 2008. – Т. 33, №1. – С. 111–115.
14. Нікіфорова Т. О. Клініко-імунологічна ефективність Протефлазиду у хворих на Епштейна-Барр-вірусну інфекцію / Т. О. Нікіфорова, В. Ф. Пюрік, О. Б. Дикий, Т. З. Кобрін // Матеріали наук.- практ. конф. та пленуму Асоціації інфекціоністів України. – Тернопіль, 2004. – С. 158.
15. Нові підходи до профілактики та терапії гострих респіраторів інфекцій у дітей раннього віку, які проживають у будинках дитини / Юліш Є. І., І. В. Баличевцева, С. Г. Гадецька [та ін.] // Суч. педіатрія. – 2009. – Т. 25, №3. – С. 15–19.
16. Досвід застосування Протефлазиду в комплексній терапії нейроінфекції у дітей / Т. А. Крючко, І. Н. Несіна, Є. С. Кошлакова, Ю. А. Костейко // Імунол. та алергол. – 2002. – №3. – С. 60.
17. Особливості терапії рецидивуючого обструктивного бронхіту у дітей раннього віку, інфікованих внутрішньоклітинними патогенами / Юліш Є. І., І. В. Баличевцева, С. Г. Гадецька, С. І. Вакуленко // Суч. педіатрія. – 2007. – Т. 16, №3. – С. 175–178.
18. Оцінка ефективності та переносимості препарату «Флавозід» у дітей з гострою респіраторною вірусною інфекцією з розвитком обструктивного синдрому: звіт. – К., 2008. – 25 с.

19. Оцінка ефективності та переносимості препарату Флавозід у дітей з герпесвірусними інфекціями: звіт. – К., 2008. – 76 с.
20. Оцінка ефективності та переносимості препарату Флавозід у дітей з герпесвірусними інфекціями: звіт. – К., 2007. – 50 с.
21. Січенко П. І. Застосування імунофлазиду у лікуванні ГРВІ у дітей раннього віку / П. І. Січенко, Н. П. Куроп'ятник // Актуальні питання клінічної медицини. – Суми: СумДУ, 2009. – С. 88.
22. Стан імунного статусу та ефективність Протефлазиду у комплексній терапії дітей з бронхолегеневою патологією, асоційованою з TORCH-інфекцією / Ф. М. Шамсієв, Н. Х. Мірсаліхова, К. І. Алімова, Д. П. Таджиханова // Суч. педіатрія. – 2011. – Т. 36, №2. – С. 39–41.
23. Токарчук Н. І. Використання Імунофлазиду для профілактики та лікування грипу і ГРВІ у дітей під час сезонного підвищення захворюваності. / Токарчук Н. І., Л. С. Старинець // Суч. педіатрія. – 2012. – Т. 41, №1. – С. 123–127.
24. Турлібеков С. С. Протефлазид у комплексній терапії вродженої цитомегаловірусної інфекції / С. С. Турлібеков // Валеологія. Астана. – 2011. – №2. – С. 164–166.
25. Харченко Ю. П. Застосування препарату Флавозід при інфекційному мононуклеозі у дітей / Ю. П. Харченко, Г. А. Шаповалова // Суч. педіатрія. – 2007. – Т. 17, №4. – С. 115–118.
26. Чернишова О. Є. Лікування та реабілітація дітей з різним перебігом герпесвірусної інфекції / О. Є. Чернишова // Внутрішньоклітинні інфекції та стан здоров'я дітей у XXI столітті: матеріали наук.-практ. конф. – Донецьк, 2005. – С. 9.
27. Шаповалова І. А. Застосування Протефлазиду в комплексній терапії та імунореабілітації підлітків з ангінами змішаної вірусно-бактеріальної етіології / І. А. Шаповалова // Проблеми екологічної та медичної генетики та клінічної імунології: зб. наук. пр. – Вип. 5 (44). – До., Луганськ; Х., 2002. – С. 106–113.
28. Юліш Є. І. Тривалий субфебрилітет у дітей. Можливі причини та підходи до терапії / Юліш Є. І., О. Є. Чернишова // Суч. педіатрія. – 2011. – Т. 35. – №1. – С. 67–72.
29. Юліш Є. І. Персистуючі герпесвірусні інфекції та їх роль у захворюваності дітей з групи часто і довго хворіючих / Юліш Є. І., С. Я. Ярошенко // Здоров'я дитини. – 2013. – Т. 50, №7. – С. 145–150.

Reference: S. O. Kramarev, O. Y. Hrynevych, O. B. Tonkovid et. al. *Meta-analysis of clinical studies on the efficacy of flavonoids in viral and viral-bacterial diseases in children* // *Modern Pediatrics*. – 2014. – No.5 (61). – pp. 1–7.

4.4. PROTEFLAZID® AND LOCAL IMMUNITY IN DISEASES CAUSED BY HUMAN PAPILLOMAVIRUS, HERPESVIRUS AND MIXED UROGENITAL INFECTIONS

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Abstract

Reporting of clinical trials results for PROTEFLAZID® in the drug formulation suppositories and vaginal swabs soaked in the solution of the drug to the local immunity of the female reproductive tract. The trials involved 216 women with viral sexually transmitted diseases: Cervical Dysplasia associated with papillomavirus infection (HPV) (Group 1); Herpes genitalis type 1 (HSV-1) and type 2 (HSV-1) (Group 2); mixed infection – HSV-1, HSV-2 and chlamydia (Group 3). Treatment results have confirmed that PROTEFLAZID® contributes to sustainable performance improvement of basic factors of local immunity – sIgA, lysozyme and C₃-complement component in the cervical mucus for all three groups of women.

Key words: local immunity, papillomavirus, herpes, mixed infection, PROTEFLAZID®.

Introduction

Local immunity protects the skin and mucous membranes of the human body from the damaging effects of viruses, bacteria, toxins, allergens, parasites, protozoa and other harmful factors. Innate immunity is carried out by local barrier properties of skin and mucous membranes, production of antimicrobial substances by them, normal microflora of an organ or tissue, phagocytic reaction, as well as removal of the damaging agent by mechanical way or by enzymatic cleavage. Mucous are characterized by the developed lymphoid tissue, and high saturation of immunocompetent cells. The immune system of mucous of the female reproductive tract can greatly resist against the introduction of pathogens, allogeneic sperm and immunologically alien fetus. Both innate and acquired immunity are of great importance.

Mucous epithelial cells are not only a physical barrier against pathogens and infectious agents, but they also secrete a wide range of protective factors, such as lysozyme, lactoferrin,

peroxidase, defensins, complement components, and cytokines and chemokines, that attract and activate immune cells [1]. Local immune urogenital system differs from other common mucous with the systematic organization. Antibodies in the female reproductive tract can be produced locally by resident plasma cells, as well as originated from the blood plasma, which the fact reflects their structural heterogeneity. Moreover, the distribution and properties of immune cells are influenced by hormonal factors [13]. Compared to other mucous secrets, for example, in rectal swabs, the female reproductive tract secretions contain relatively high levels of immunoglobulins. The main type of immunoglobulins involved in local immunity, especially in the maintenance of normal microflora, is secretory immunoglobulin A (sIgA). By contacting microorganisms, it inhibits their adherence to the cell surface; and, together with nonspecific immunity factors, it protects mucous membranes against microorganisms and viruses. This immunoglobulin has an additional secretory component, which is synthesized by epithelial cells of the mucous membranes. It attaches two IgA molecules during the passage of the latter through the epithelial cells. Integral sIgA is considerably more resistant to proteolytic enzymes even in the gastric content. The majority of mucous secretions (intestinal swabs, tears, saliva, milk) show the dominance level of sIgA among other immunoglobulins [4,7].

Lysozyme is synthesized by neutrophils, it is found in cervico-vaginal fluid and, to a greater concentration, in the cervical mucus plug [3,6,8,16]. Being an enzyme of the hydrolase class, it has the property of lysing peptidoglycan (murein) on the cell wall of Gram-positive bacteria, such as streptococci. Furthermore, lysozyme can destroy bacteria by using non-enzymatic mechanisms. In addition to the antibacterial effect, the ability of lysozyme to block the introduction of viruses into the cell and further replication was highlighted [2,12].

Activation of complement component C_3 supports phagocytosis, enhances vascular permeability, leukocyte chemotaxis and antigen-antibody compound. A characteristic feature of reactions of the complement activation is that every product from the previous reaction is a catalyst for the further stage of activation, due to which there is a multiple strengthening of impact of primary stimulus. Immediately after the activation of the complement system, opsonizing components that cover pathogens or immune complexes are formed by attracting phagocytes. Their presence on the surface of phagocytic cells enhances their attachment to the opsonized bacteria and activates the absorption process. C_3 is the central component of the complement system, and the acute phase protein. This is the most important part of the defense system against foreign agents; it consists of about 70% of all the proteins of the complement system. Complement component C_3 is involved in both routes, namely the classical and the alternative pathway of the complement activation. In the classic pathway, its formation is activated by IgG and IgM, in the alternative pathway – by toxins, including endotoxin and IgA. C_3 activation supports phagocytosis, enhances vascular permeability, increases the contraction of smooth muscle, leukocyte chemotaxis and antigen-antibody compound. Along with other organs, it is synthesized by epithelial cells of the mucous of the female reproductive system. C_3 content is reduced due to its consumption at the classical and alternative pathways of the complement system activation, in particular locally in the case of long-term infections of the female genital tract [13].

Summarizing the importance of local immunity factors, it should be emphasized that the reduction of sIgA, lysozyme and complement component C_3 in female genital tract indicates

insecurity of mucosal epithelium, weakening of local resistance, and leads to activation of various infectious agents. The latter, in turn, is responsible for disruptions of vaginal microbiota (biocenosis) and the emergence of various lesions of mucous membranes of the genital tract of women with typical clinical manifestations in each case.

The drug Proteflazid® is applied as a liquid formulation (extract in the form of droplets), or in the form of suppositories, it contains flavonoids derived from a mixture (1:1) of herbs *Deschampsia caespitosa* L. and *Calamagrostis epigeios* L. The active substance of the drug (flavonoids) inhibits the synthesis of DNA and RNA viruses in infected cells through the suppression of activity of virus specific enzymes of RNA and DNA polymerases, thymidine kinase, reverse transcriptase; it has immunotropic properties. According to several authors, the drug Proteflazid® is an effective mean in infectious diseases of the female genitals [9,10,17-23].

The aim of the study

Examine the state of local immunity in the reproductive tract of women with sexually transmitted diseases caused by human papillomavirus, herpes viruses (Type 1, 2) and mixed infection (herpes viruses + chlamydia). To compare dynamic changes in the performance of sIgA, lysozyme and complement component C₃ in the treatment with two forms of the drug Proteflazid®: in the traditional form of tampons soaked with the solution of the drug, and in the new medicinal form of suppositories.

Materials and methods

The study was conducted from October 2013 to December 2014 at the Department of Obstetrics, Gynecology and Reproduction of Shupyk National Medical Academy of Postgraduate Education, on the basis of Kiev City Center for Reproductive and Perinatal Medicine; on the basis of the rehabilitation department of reproductive function of women at the SE „Institute of Pediatrics, Obstetrics and Gynecology of NAMS of Ukraine”, as well as at the department of obstetrics and gynecology No.3 of the Bogomolets National Medical University, on the basis of the day hospital of Kiev City Clinical Maternity Hospital No.3. According to the design, the study is characterized as an open, controlled, randomized, study with parallel-design. The study included 216 women with sexually transmitted diseases. The randomization was stratified according to the treatment with vaginal suppositories containing the drug Proteflazid®, and with vaginal swabs soaked in the solution of Proteflazid® extract. 108 patients received Proteflazid® suppositories, and tampons soaked in the solution of the drug Proteflazid® were administered to 108 patients, too. Criteria for the inclusion in the study: group 1 – 76 patients with cervical dysplasia and HPV (Human papillomavirus); group 2 – 70 patients infected with herpes simplex virus types 1 and 2 (HSV-1, HSV-2); group 3 – 70 patients with mixed urogenital infections, such as herpes infection (HSV1, HSV-2) and chlamydia (Chlamydia). Patients of all three groups were divided into 2 subgroups: subgroup A consisted of patients treated with vaginal suppositories containing the drug Proteflazid® (“Farmeks Group”, Ukraine) and subgroup B consisted of patients treated with vaginal swabs containing the solution of Proteflazid® extract (“Phytopharm”, Ukraine; 3 ml of the drug in 20 ml of isotonic solution). Both formulations of the drug Proteflazid® were used twice a day for 14 days in patients of group 1 and 3, and for 10 days – in patients of group 2. Patients of both subgroups of group 2 also took acyclovir (Gerpevir, “Kyivmedpreparat”, Ukraine), 200 mg tablets, 1 tablet 5 times a day, for 5 days. Patients of both subgroups of Group 3 also took azithromycin (Azimed, “Arterium”, Ukraine), 500 mg tablets, according to the scheme: 1 day of treatment – 1.0 g, 2-5 day of treatment – 0.5 g per day. Indexes of nominally healthy women (patient at a dispensary observation in the state of relatively complete remission of diseases caused by HPV, and HSV-1, HSV-2) served as control (n=10).

Methodology for assessment of local immunity.

Mucus from the cervical canal was collected with cytobrush into Eppendorf tube, the tube was placed in the freezer at -20°C and kept until the study conduct. Before the study, after thawing and adding buffer solution, the supernatant was extracted and collected with the help of agitating and centrifugation. In the prepared cervical mucus samples the content of sIgA, C_3 component complement and lysozyme was determined by enzyme immunoassay (ELISA). Kits produced by „Immundiagnostik AG” company (Germany) were used for determining the concentration of sIgA and lysozyme, and a kit produced by „Assaypro LLC” company (USA) was used for determining the concentration of C_3 complement component. Optical density was measured on tablet analyzer „Multiscan PLUS” at a wavelength of 450 nm.

The quantitative content of sIgA, lysozyme and complement component C_3 in cervical mucus was normalized in terms of total protein in the samples. Protein was determined by the method based on the biuret reaction, by using a set of „LiquickCor – TOTALPROTEIN 60” („CORMAY”, Poland). Optical density was measured on tablet analyzer „Multiscan PLUS” at a wavelength of 450 nm.

Calculation of the results was performed through the Excel program (Microsoft Office Package). Results for sIgA, lysozyme and complement component C_3 concentrations were expressed in ng/ml with the subsequent conversion into mg/l. The results for total protein were obtained in g/l, with the subsequent conversion into mcg/g of protein.

Statistics

We determined arithmetic mean (M), median, standard deviation (SD), and standard error (SE). Differences in the treatment dynamics were counted with the help of the two-tailed Fisher exact test and the Mann-Whitney test. Changes at $p < 0.05$ were considered reliable.

Results

Baselines for sIgA, lysozyme and complement component C_3 in subgroups A and B (use of the drug in the form of suppositories Proteflazid® and tampons soaked in the solution of Proteflazid® extract) in patients of group I with certain disease “Dysplasia of cervical epithelium, infection caused by the human papillomavirus (HPV)” differed insignificantly or tended to decrease, compared to indexes of nominally healthy women. After the course of treatment with the drug Proteflazid®, the levels of sIgA, lysozyme and complement component C_3 significantly increased in both groups, and remained at elevated levels after the treatment, compared to baselines and to similar indexes in the group of nominally healthy women (fig. 1a, 1b, 1c).

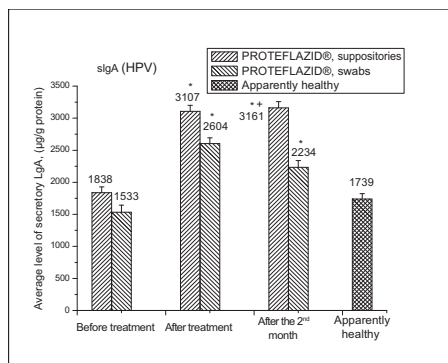


Fig. 1a

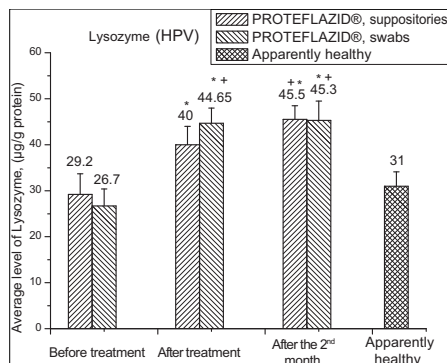


Fig. 1b

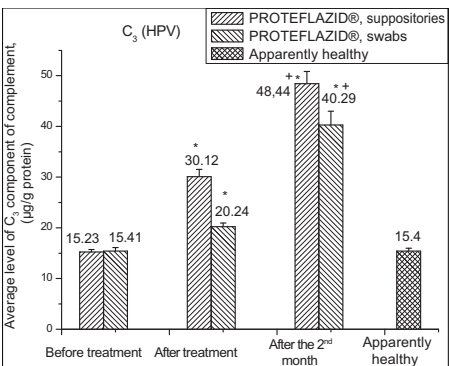


Fig. 1c

Figure 1. Dynamics of sIgA (1a), lysozyme (1b) and complement component C₃ (1c) in patients with “cervical dysplasia epithelium caused by HPV infection”, before and after treatment with the drug Proteflazid[®], administered in the form of vaginal suppositories and vaginal swabs soaked in the solution of Proteflazid[®] extract (mean ± SE)
* – p<0.05 against the level before treatment
+ – p<0.05 against the conventional norm

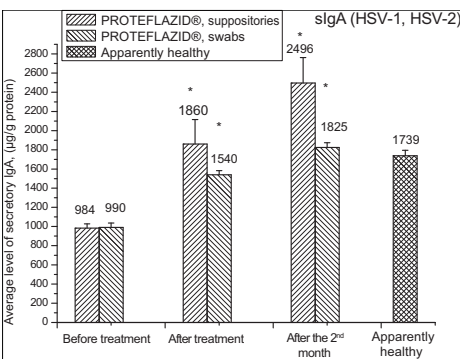


Fig. 2a

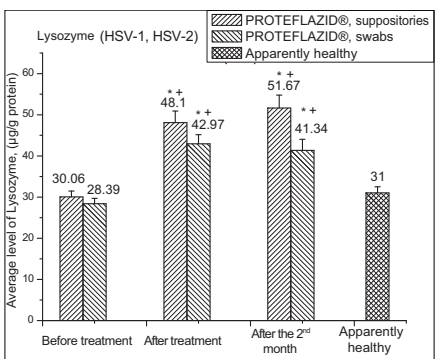


Fig. 2b

Figure 2. Dynamics of sIgA (2a), lysozyme (2b) and complement component C₃ (2c) in patients with “Genital Herpes (HSV-1, HSV-2) in the exacerbation phase”, before and after treatment with the drug Proteflazid[®], administered in the form of vaginal suppositories and vaginal swabs soaked in the solution of Proteflazid[®] extract (mean ± SE)
* – p<0.05 against the level before treatment
+ – p<0.05 against the conventional norm

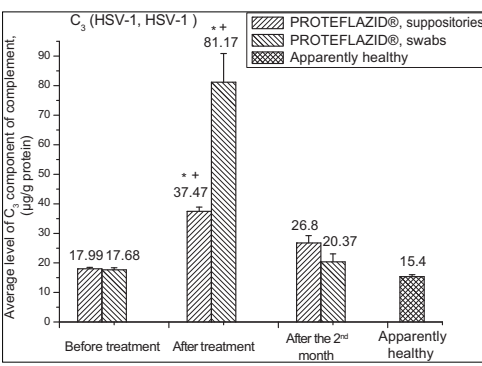


Fig. 2c

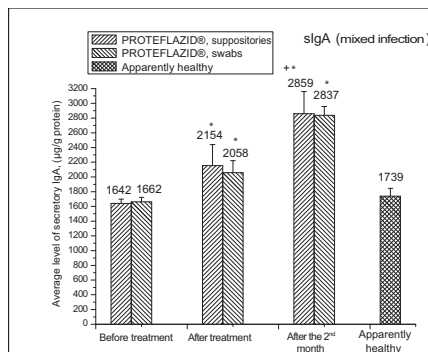


Fig. 3a

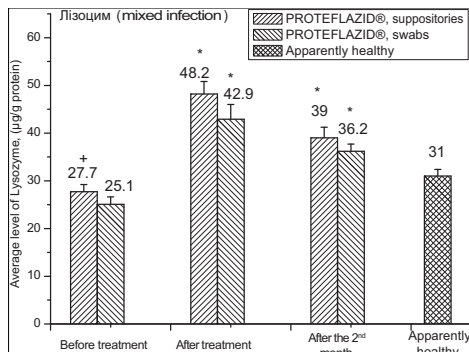


Fig. 3b

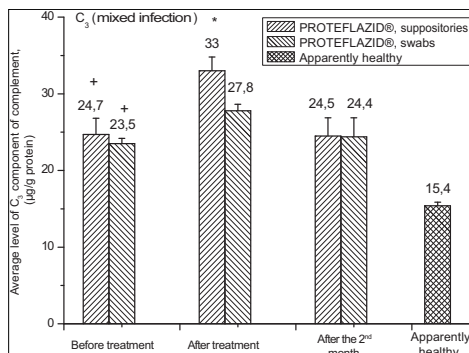


Fig. 3c

Figure 3. Dynamics of sIgA (3a), lysozyme (3b) and complement component C₃ (3c) in patients with “Urogenital viral and bacterial infection (Herpes simplex viruses and Chlamydia)”, before and after treatment with the drug Proteflazid®, administered in the form of vaginal suppositories and vaginal swabs soaked in the solution of Proteflazid® extract (mean ± SE)

* – $p < 0.05$ against the level before treatment

+ – $p < 0.05$ against the conventional norm

sIgA baselines in subgroups A and B (before treatment) in patients in group 2 with certain disease “Genital Herpes (HSV1, HSV-2) in the exacerbation phase” were lower compared to the conventional norm. After treatment, sIgA indexes increased significantly in both groups; and in subgroup A, where the drug Proteflazid® was used in the form of suppositories, the increase was even more pronounced. It should be noted that the significant increase of sIgA level after treatment allowed to reaching the level of conventional norm. 2 months after the end of treatment, sIgA level remained high. Increase in indexes was more pronounced in the subgroup where suppositories (fig.2a) were used. Lysozyme baseline in subgroups A and B of patients of group 2 was slightly lower than the conventional norm; it increased significantly immediately after treatment in both groups, while remaining at a stable high level after the end of treatment (fig.2b).

Level of complement component C₃ before treatment was slightly higher than normal, and it increased considerably after the treatment, that increase was more significant in subgroup B. Two months after treatment, level of complement component C₃ decreased almost to the baseline, while remaining slightly higher in the subgroup A (fig.2c). When compared with the conventional standard, the level of lysozyme increased significantly immediately after treatment and two months after treatment, while the increase of complement component C₃ level compared to conventionally normal levels was noted after treatment.

In subgroups A and B of Group 3 in patients with certain disease “Urogenital viral and bacterial infection (herpes simplex viruses and chlamydia)” sIgA level was lower than conventional levels before treatment with the drug Proteflazid®. After treatment, the sIgA level increased significantly. Two months after treatment, sIgA level increased to a greater extent, and sIgA level in subgroup A was significantly higher than conventionally normal level (fig. 3a). Lysozyme level before treatment in subgroups A and B was slightly lower than the conventional norm. After treatment with the drug Proteflazid®, lysozyme level significantly increased in both groups. After 2 months after treatment lysozyme level remained higher than before treatment (fig. 3b). Level of complement component C₃ before treatment was significantly higher than the conventional norm, that is, there was activation of the complement system due to the fact that the aggravation of infection was caused by a mixed infection in which not only herpes simplex virus, but also chlamydia were revealed. However, lower indexes of real antiinfective factors of nonspecific protection, such as sIgA and lysozyme, were observed. Such dysregulation of natural protection factors reflects the lack of adequate confrontation against the aggravation of infection. After treatment with the drug Proteflazid®, the level of complement component C in both groups (A and B) increased, but the increase was significant only in the subgroup A, wherein the drug Proteflazid® was used in the form of suppositories. 2 months after treatment, the level of complement component C₃ was back to the baseline (fig. 3c).

Discussion

Our studies confirmed the lack of response of the local immune system in diseases associated with HPV infection, genital herpes, and mixed infection (herpes viruses and chlamydia) in almost all the surveyed patients. It is due to the fact that sIgA, lysozyme and complement component C₃ levels in the cervical mucus before treatment were close or tended to be less than similar indexes in nominally healthy women. Lower values of the indexes were noted in women with genital herpes. This situation is typical for slow chronic inflammatory process, when the detected imbalance in the local protective factors is not easy to recover in the course of treatment. The results indicate that the infectious process is accompanied by suppression of immune and protective properties of vaginal mucus in women, as evidenced by a significant reduction in secretory immunoglobulin A and lysozyme. Reducing the sIgA concentration in cervical mucus in women is closely linked to infectious and inflammatory genital diseases. Accordingly, infectious factors, along with other exogenous factors, such as reduction in resistance of the normal vaginal microflora, hormonal changes, cause low resistance of a woman against infectious agents and contribute to the aggravation of persistent infections.

Women with disruptions in the vaginal ecosystem become more susceptible to sexually transmitted diseases [14]. Several mechanisms oppose the spread of infectious agents, the most significant of which are the integrity of the mucosa, protective mucus, acidity, commensal bacteria, local and systemic immunity. The immune response of the mucosa in the genital tract plays an important role in preventing the invasion and the proliferation of microorganisms. Vaginal epithelial cells and resident macrophages recognize changes in tissue homeostasis, and activate neutrophils, macrophages, dendritic cells and natural killer cells [5,11,15]. In cases where immediate innate immune system is unable to fight against the spread of infection, it sends signals to acquired immunity for the activation of T and B lymphocytes [5,11]. In clinical practice, as a rule, they carry out typing of microorganisms involved in the process of vaginal infection, while ignoring however vaginal status according to immune response and, first of all, innate mucosal immunity state. Meanwhile, mucosal immunity is the first mucosal protection and reflects largely the state of vaginal homeostasis.

Our study showed that the drug Proteflazid® in the treatment of sexually transmitted diseases significantly improves such indexes of local immunity as sIgA, lysozyme and complement component C₃ in the cervical mucus. The improvement is stable and maintained for a long time after treatment. Moreover, the drug contributes to the correction of deregulated factors of natural protection during exacerbation of mixed infections, when due to lower levels of sIgA and lysozyme activation of complement component C₃ can be observed. After treatment with the drug, particularly at the use of suppositories Proteflazid®, an increase was observed in all three components of the natural protection, and ratios between them were aligned to optimum values, at which the most significant anti-infective protection can be realized.

The relationship between macroand micro-organism in vaginal homeostasis is complex. It is hard to imagine that a single protective factor may prevent the emergence and the development of infection in a host organism. Only a set of protective factors inherent in the body and being in an active state can really resist infection. That is, “aggregation” (in this case, the process of combining components into one system) and mobilization of protective factors can actually withstand the local spread of infection. This may explain the positive effect of the drug Proteflazid® if administered locally for the treatment of sexually transmitted diseases, when after a course of treatment with the drug such levels of the main protective factors as sIgA, lysozyme and complement component C₃ increase significantly and substantially in the cervical mucus.

Conclusions

The drug Proteflazid® in the form of suppositories and vaginal swabs soaked in the solution of the drug significantly and consistently improves the content and improves the ratio of such major factors of local immunity as sIgA, lysozyme and complement component C₃, and strengthens thus anti-infectious protection of the cervical mucus in the female reproductive system as a whole in the treatment of sexually transmitted diseases and infections caused by human papillomavirus, herpes viruses and mixed urogenital infections (Herpes viruses + Chlamydia).

References:

1. Amjadi F., Salehi E., Medhdizadeh M. et al.: Role of the innate immunity in female reproductive tract. *Adv. Biomed. Res.*, 2014; 3: 1–15.
2. Cole A. M., Cole A. L.: Antimicrobial polypeptides are key anti-HIV-1 effector molecules of cervico-vaginal host defense. *Am J Reprod Immunol*, 2008; 59: 27–34.
3. Cole A. M.: Innate host defense of human vaginal and cervical mucosae. *Curr Top Microbiol Immunol*, 2006; 306: 199–230.
4. Crowley-Nowick P. A., Bell M., Edwards R. P. et al.: Normal uterine cervix: characterization of isolated lymphocyte phenotypes and immunoglobulin secretion. *Am J Reprod Immunol*, 1995; 34: 214–247.
5. Fahey J. V., Sentman C. L., Pioli P. A. et al.: Innate and adaptive immunity in female genital tract: cellular responses and interactions. *Immunol Rev*, 2005; 206: 306–335.
6. Hein M., Valore E. V., Helmig R. B. et al.: Antimicrobial factors in cervical mucus plug. *Am J Obstet Gynecol*, 2002; 187: 137–44.
7. Jackson S., Mestecky J., Moldoveanu Z. et al.: Appendix I: collection and processing of human mucosal secretions. – in – *Mucosal Immunology*, 3rd edn. Amsterdam, The Netherlands, Elsevier Academic Press, 2005: 1647–1659.
8. Janeway C. A., Jr, Medzhitov R.: Innate immune recognition. *Ann Rev Immunol*, 2002; 20: 197–216.
9. Kaminsky V. V., Litus A. I., Grynevych O. Y. et al.: Proteflazid®: Efficacy and safety in urogenital herpes virus infection (systematic review). *Science and Education Studies*, 2015; 2(16): 705–727.

10. Kornatska A., Revenko O., Grynevych O. Y. et al.: Therapeutic efficacy and tolerability profiles of Proteflazid®, suppository and drops among the patients with herpesvirus infection in the exacerbation phase. *European Journal of Scientific Research*, 2016, 1(9): 506–522.
11. Ledger W. J., Witkin S. S.: Vaginal immunity. – in – Ledger W. J., Witkin S. S. (eds.): *Vulvovaginal infections*, Washington, ASM Press, 2007; 12–15.
12. Lee-Huang S., Maiorov V., Huang P. L. et al.: Structural and functional modeling of human lysozyme reveals a unique nona peptide, HL9, with anti-HIV activity. *Biochemistry*, 2005; 44: 4648–4655.
13. Russell M. W., Mestecky J.: Humoral immune responses to microbial infections in the genital tract. *Microbes and infection*, 2002; 4: 667–677.
14. Sewankambo N., Gray R. H., Wawer M. J. et al.: HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. *Lancet*, 1997; 350: 546–550.
15. Soehnlein O., Lindbom L.: Phagocyte partnership during the onset and resolution on inflammation. *Nature Rev. Immunol*, 2010; 10: 427–439.
16. Swart P. J., Kuipers E. M., Smit C. et al.: Antiviral activity of lactoferrin. *Adv Exp Med Biol*, 1998; 443: 205–12.
17. Баєв А. І., Кузієва Г. Д., Вуйко І. В.: Обґрунтування застосування Протефлазиду в комплексній терапії мікст – ІПСШ (Повідомлення 4). *Питання Дерматології та Венерології*, 2011; 3–4 (47–48): 34–37.
18. Баєв А. І., Кузієва Г. Д., Сілаєв Д. В. та ін: Обґрунтування застосування Протефлазиду в комплексній терапії мікст-ІПСШ (Повідомлення 3). *Питання Дерматології та Венерології*, 2011; 3–4 (47–48): 31–33.
19. Бенюк В. О., Гриневич О. Й., Усевич І. А. та ін: Терапевтична ефективність супозиторіїв ПРОТЕФЛАЗІД® при змішаній урогенітальній вірусно-бактеріальній інфекції. *Здоров'я жінки*, 2016, 2 (106): 162–166.
20. Вовк І. Б., Ревенько О. А., Данилюк О. І.: Використання флавоноїдів у комплексному лікуванні жінок із запальними захворюваннями геніталій вірусно-бактеріальної етіології. *Здоров'я жінки*, 2002; 4(12): 43–45.
21. Годлевська Н. А., Старовір О. В.: Ефективність системного та місцевого застосування препарату Протефлазид® у лікуванні патологій шийки матки, спричиненої папіломавірусною інфекцією. *Здоров'я жінки*, 2012; 3(69): 80–83.
22. Камінський В. В., Шалько М. М., Воробйова Л. І. та ін: Протефлазид®: специфічна активність у доклінічних дослідженнях, ефективність та безпека застосування в клінічній практиці при захворюваннях, спричинених вірусом папіломи людини. *Здоров'я жінки*, 2015 року; 3 (99): 128–138.
23. Ромащенко О. В., Руденко О. В.: Протефлазид у комплексній терапії запальних захворювань геніталій у жінок. *Педіатрія, акушерство та гінекологія*, 2002; 3: 89–94.

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Section 5

5.1. VIRAL HEPATITIS: STATISTICAL ANALYSIS OF CLINICAL RESEARCH ON THE EFFECTIVENESS OF USING THE DRUG PROTEFLAZID®

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Abstract

A search for information on clinical studies devoted to the study of the effectiveness and safety of the use of the drug PROTEFLAZID® in the treatment of viral hepatitis (B, C) was carried out on the Internet using information and search systems. As a result of the search, 8 scientific publications of the results of clinical studies on the effectiveness of the use of the drug PROTEFLAZID® in the treatment of viral hepatitis B and C in the adult category of patients (scientific works published in the period from 2000 to 2004 years). The conducted statistical analysis of the results of clinical studies shows: under the influence of the treatment of hepatitis with the drug PROTEFLAZID®, regression of cytolytic and cholestatic syndromes, restoration of protein synthetic and hemostatic functions of the liver, reduction of hepato- and splenomegaly, restoration of liver structure, reduction of the number of antigens and the intensity of viral replication in the blood is noted. Taking into account the good tolerability, high safety profile, complex effect on many stages of the development of viral hepatitis, the drug PROTEFLAZID® (drops) can be recommended as an effective and safe antiviral agent for the treatment of viral hepatitis (B, C).

Key words: PROTEFLAZID® (drops), hepatitis B, hepatitis C, adult patients, treatment, meta-analysis, t-criteria.

Introduction

Viral hepatitises (B, C) are one of the major human pathologies based on their social, economic and medical significance. They are characterized by the global prevalence, continuing trend towards the increased incidence rate, predominantly chronic forms and high mortality level in the world. At present, the viral hepatitises – in addition to the acute respiratory diseases, HIV infection and (in some regions) tuberculosis – compose a group of the crisis infections [1, 2].

Anicteric, subclinical forms of the acute and chronic HBV (CHB), like the cirrhosis, liver cancer and extrahepatic manifestations caused by hepatitis B virus (HBV) mostly remain unrecognized and are not recorded as the infectious disease. Based on the results of the

seroepidemiologic studies conducted in Ukraine, it was determined that the total detection rate of HBsAg and anti-HBs among patients of the health care institutions of different specializations is generally 28.6%, among blood donors – 15.3% and among the healthcare workers – 32.2%.

According to the global epidemiology, 500 to 700 million people are now infected with hepatitis C virus (HCV) accounting for 10% of the world's population. Approximately 200 million people are living with the chronic hepatitis C (CHC) infection. Conducted epidemiological studies indicate that Ukraine belongs to the regions with the high prevalence of HCV infection. Currently, up to 3% of the Ukrainian population is infected with HCV. Such aspects as prognosis of viral hepatitis, differential diagnosis, diagnosis of the acute and chronic viral hepatitises, liver cirrhosis and the adequate therapy still remain under-investigated.

The chronic hepatitises pose quite significant therapy challenges. Evidence shows that the basis of viral hepatitis development is the prolonged persistence of viral agents occurred as a result of the functional defects of the cellular and humoral immunity factors, blockade of specific effector reactions and the interferon genesis system [1, 2].

Therefore, the development of the new medicinal products for viral hepatitis treatment and prevention is quite relevant issue.

Most medicinal products used in the viral infections are synthetic and semisynthetic purine and pyrimidine antimetabolites, in particular, dideoxy-nucleoside analogues targeted to the viral enzymes – transcriptase and protease. There are only rare references in the literature about using the viral inhibitors of the different structure.

The search of the new chemical compounds with the combined effect that would have inhibitory properties against the viruses and would not have the immunosuppressive effect has become the actual problem. The most promising agents in this area are the herbal substances that contain spatially arranged phenols with a group of flavonoids and flavonoid-like substances, most of which are found as glycosides in plants.

PROTEFLAZID® is the antiviral product containing some flavonoid glycosides isolated from the wild grasses: *Herba Deschampsia caespitosa* L. and *Herba Calamagrostis epigeios* L.

The flavonoids contained in the medicinal product have the polypharmacological effect:

a) direct antiviral effect: they inhibit the replication of the DNA and RNA viruses. The mechanism of the antiviral effect involves the inhibition of the virus-specific enzymes – polymerases and thymidine kinase;

b) immunotropic effect: they act as the inducers of the endogenous alpha- and gamma-interferon facilitating the increased nonspecific resistance of the body to the viral and bacterial infections;

c) antioxidant effect: they inhibit the free-radical processes preventing the accumulation of the lipid peroxidation products, enhance the antioxidant status of the cells decreasing the intoxication;

d) apoptosis-modulating effect: potentiating the action of the apoptosis-inducing substances and activating caspase 9, they promote the elimination of virus-infected cells and primary prevention of the chronic diseases secondary to the latent viral infections.

PROTEFLAZID® is intended for topical and systemic use. The medicinal product prevents the recurrences and prolongs the remission period.

Purpose

To perform the statistical analysis of the results of clinical studies using the meta-analysis and t-test to evaluate the efficacy and safety of the medicinal product PROTEFLAZID® in the therapy of patients with the viral hepatitis B and C.

Materials and methods

Meta-analysis is a statistical analysis that summarizes the data from several similar studies into a single result and allows evaluating the efficacy of treatment or diagnosis using significantly larger sample size than in a separate study. The meta-analysis may mathematically combine two or more studies aimed to test the same hypothesis [1, 3].

The Fisher's z-test was used to determine the statistical significance of the pooled odds ratio. In meta-analysis, the heterogeneity test was used as the usual method of assessing whether a dataset of single studies is homogeneous. The χ -squared test was used to assess the between-study heterogeneity. $P < 0.05$ indicated the statistically significant heterogeneity. Also, the quantitative evaluation of the heterogeneity effects was performed using I^2 -test representing the proportion of variability across studies. If $P < 0.05$ in the χ -squared test or the result of I^2 -test is more than 50%, it indicates the heterogeneity across the studies and then the random effects model (DerSimonian and Laird method) is selected for meta-analysis. In other case, the fixed effect model (Mantel-Haenszel method) is used.

The analysis of outcomes was performed using the Forest plot. Plot header informs which comparison is displayed in the diagram and which type of outcome is evaluated in each study. The vertical line in the middle ($OR = 1$) represents the point where there is no difference in the outcome between the treatment and control groups. Box of each specific study (Blob) has larger size than the statistical weight (Weight%) of this study in the overall pool. The horizontal lines ("whiskers") near the box of each study represent the confidence interval (95% CI) of the study. The diamond represents the pooled result where the diamond length is the pooled estimate of 95% CI. The lower horizontal axis estimates the treatment effect compared with control. The left side of "1" vertical line corresponds to the decreased odds ratio in the treatment versus control. The right side of "1" vertical line suggests the increased odds ratio during the treatment versus control.

It is also reasonable to perform the sensitivity analysis to estimate the quality and consistency of results omitting each study during the analysis. Funnel plot (sample size) is used to assess the publication bias. The shorter confidence interval for a particular study, the more precise its estimate of the treatment effect – the higher this study is appeared on the plot. Deviation from a funnel distribution indicates the presence of the systematic error in the study sample.

If number of patients was specified in percents, then t-test was used. Test significance greater than 0.05 indicates that the means do not differ from each other. Test significance lower than 0.05 indicates that the means significantly differ from each other.

RevMan software tool was used to perform the meta-analysis and IBM SPSS software package was used to do the t-test.

Data sources

The study was conducted based on the search for information about the clinical studies devoted to the investigation of the efficacy and safety of medicinal product PROTEFLAZID® used in treatment of viral hepatitis (B and C) in Internet using the information retrieval systems, reports and publications.

Upon the search, we selected 8 publications of the results of clinical studies of efficacy of medicinal product PROTEFLAZID® used in treatment of viral hepatitis B and C in the adult patients.

All scientific papers outlining the efficacy of medicinal product PROTEFLAZID® used in treatment of viral hepatitis B and C were published in 2000–2004.

List of the publications presenting the efficacy of medicinal product PROTEFLAZID® used in treatment of viral hepatitis B and C in patients that were selected for the statistical analysis are provided in Table 1.

Table 1. List of publications presenting the efficacy of medicinal product PROTEFLAZID® used in treatment of viral hepatitis B and C in patients

Author	Total number of patients	Area of PROTEFLAZID® use	Results of clinical studies
Dykyi B. M. [4]	37	Viral hepatitis B	Reduction of manifestations of intrahepatic cholestasis, normalization of the blood enzyme range, reduction of lipid peroxidation processes.
Nartov P. V. [5]	35	Acute viral hepatitis B	Normalization of ALT activity levels, significant reduction of hepatitis B virus replication.
Ivakhiv O. L. [6]	56	Chronic viral hepatitis B and C	Faster onset of clinical and biochemical remission, shorter hospital stay.
Matiash V. I., Shevchuk V. B. [7]	45	Viral hepatitis B and C	Positive changes of restoration of hepatic homeostatic functions (detoxification, excretory, protein synthesis) over time, relief of cholestatic and cytolytic syndromes (decreased bilirubin levels, ALT and AST activity).
Matiash V. I., Bobrova I. A. [8]	40	Acute and chronic viral hepatitis B	Positive effect on the clinical course of diseases, decreased intensity of cytolytic syndrome, autoimmune processes, virus replication activity, restoration of liver structure.
Matiash V. I. [9]	45	Viral hepatitis B and C	Faster regression of disease parameters, restoration of the hepatic functional state, its detoxification activity, relief of cholestatic and cytolytic syndromes, normalization of blood hyperbilirubinemia, ALT activity.
Matiash V. I., Bobrova I. A., Shevchuk V. B., Tokunova T. L. [10]	85	Chronic viral hepatitis B and C	Positive effect on the restoration of the structural and functional state of the affected liver and its homeostatic functions (detoxification, excretory, protein synthesis).
Matiash V. I. [11]	120	Viral hepatitis B and C	Persistent positive effect with normalization of transferase activity, absence of HBeAg antigenemia and blood viremia.

We selected 8 publications of the results of clinical studies of efficacy of medicinal product PROTEFLAZID® used in treatment of 463 patients with viral hepatitis B and C.

Patients had thorough pre-and post-treatment examination involving the generally accepted clinical and laboratory-instrumental methods, in particular, ultrasound examination of the liver and spleen, examination of the cellular and humoral immunity parameters, Doppler imaging. The polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) were used as the main diagnostic methods.

Study results

Statistical analysis of the clinical studies reflecting the results of efficacy and safety of the medicinal product PROTEFLAZID® used in the treatment of viral hepatitis B and C was performed by the following statistically significant parameters:

I. Changes of the blood biochemical parameters in patients with the acute and chronic viral hepatitis B and acute and chronic hepatitis C secondary to administration of PROTEFLAZID®: 1. Total bilirubin, $\mu\text{mol/l}$. 2. ALT activity in whole and diluted blood, $\text{mmol/l} \cdot \text{h}$. 3. AST activity in whole and diluted blood, $\text{mmol/l} \cdot \text{h}$. 4. Total protein, g/l . 5. α_1 -globulins, %. 6. α_2 -globulins, %. 7. γ -globulins, %. 8. Circulating immune complexes, U. 9. Prothrombin index, %.

II. Changes of the sonographic signs of the hepatic injury in patients with the acute and chronic viral hepatitis B and acute and chronic hepatitis C secondary to administration of PROTEFLAZID®: 1. Enlarged liver, cm. 2. Enlarged spleen, cm. 3. Rate of increased liver echogenicity secondary to the treatment with PROTEFLAZID® during 6 months. 4. Rate of increased liver echogenicity 3 months after treatment with PROTEFLAZID®. 5. Rate of the fine granular liver structure 3 months after treatment with PROTEFLAZID®.

III. Rate of detection of the serological markers of viral hepatitis B: 1. Rate of detection of the HBV markers (Ag HBc) secondary to the treatment with PROTEFLAZID® during 1 month. 2. Rate of presence of HBV markers (IgM HBc) secondary to the treatment with PROTEFLAZID® during 3 months. 3. Rate of presence of HBV markers (IgM HBc) secondary to the treatment with PROTEFLAZID® during 6 months. 4. Rate of presence of HBV DNA in blood by PCR method secondary to the treatment with PROTEFLAZID® during 3 months. 5. Rate of presence of HBV DNA in blood by PCR method secondary to the treatment with PROTEFLAZID® during 6 months.

Analysis of the clinical studies based on t-test.

The results of the clinical studies analyzed based on t-test are provided in Table 2.

Table 2. Evaluation of results of clinical studies of efficacy of medicinal product PROTEFLAZID® used in treatment of viral hepatitis B and C in patients based on t-test

#	Parameter	Reference value	Pre-treatment mean	Post-treatment mean			p-value
				1 month	2 months	3 months	
1.	Total bilirubin, $\mu\text{mol/l}$	8.5–20.5	140.1	28.55	18.23 (normal)	14.08 (normal)	< 0.05 (1 month after)

2.	ALT w, mmol/l · hour	0.1–0.68	4.15	1.00	0.63 (normal)	0.61 (normal)	< 0.05 (1 month after)
3.	ALT d, mmol/l · hour	0.1–0.68	8.85	0.5 (normal)	0.2 (normal)	0.18 (normal)	< 0.05 (1 month after)
4.	AST w, mmol/l · hour	0.1– 0.45	2.48	0.43 (normal)	0.33 (normal)	0.18 (normal)	< 0.05 (1 month after)
5.	AST d, mmol/l · hour	0.1–0.45	2.23	0.15 (normal)	0.10 (normal)	0.13 (normal)	< 0.05 (1 month after)
6.	Total protein, g/l	65–85	66.40 (normal)	66.85 (normal)	68.87 (normal)	70.28 (normal)	> 0.05
7.	α 1-globulins, %	3.6–5.6	4.80 (normal)	4.93 (normal)	5.40 (normal)	5.35 (normal)	> 0.05
8.	α 2-globulins, %	6.9–10.5	8.57 (normal)	8.87 (normal)	8.60 (normal)	8.55 (normal)	> 0.05
9.	γ -globulins, %	15–22	22.33	21.50 (normal)	22.83	20.40 (normal)	> 0.05
10.	Circulating immune complexes, U	< 75	72.9 (normal)	51.7 (normal)	43.3 (normal)	31.1 (normal)	< 0.05 (1 month after)
11.	Prothrombin index, %	78–142	70.05	72.10	76.30	79.38 (normal)	< 0.05 (2 months after)
12.	Enlarged liver, cm	0	2.93	2.73	2.37	2.00	< 0.05 (3 months after)
13.	Enlarged spleen, cm	0	1.70	1.53	1.40	1.15	> 0.05

Source: Formed on the basis of [11] (80 patients with AHB and CHB were examined, 40 of them received PROTEFLAZID®, another 40 received PROTEFLAZID® in combination with plasmapheresis).

Total bilirubin was within normal after 2-month treatment with medicinal product PROTEFLAZID®. However, mean total bilirubin level was decreased. Significance of t-test (less than 0.05) indicates that the mean total bilirubin level in adult patients who received PROTEFLAZID® is significantly different from the pre-treatment mean total bilirubin level in adult patients starting already from the first month of treatment.

ALT in whole blood was within normal after 2-month treatment with medicinal product PROTEFLAZID®. Mean ALT w was decreased. Significance of t-test (less than 0.05) indicates that the mean ALT w in patients who received PROTEFLAZID® is significantly different from the pre-treatment mean ALT starting already from the first month of treatment.

ALT in diluted blood was within normal after 1-month treatment with medicinal product PROTEFLAZID®. Mean ALT d was decreased. Significance of t-test (greater than 0.05) indicates that the mean ALT in patients who received PROTEFLAZID® is significantly different from the pre-treatment mean ALT starting already from the first month of treatment.

AST in whole blood was within normal after 1-month treatment with medicinal product PROTEFLAZID®. Mean AST w was decreased. Significance of t-test (less than 0.05) indicates that the mean AST w in patients who received PROTEFLAZID® is significantly different from the pre-treatment mean AST starting already from the first month of treatment.

AST d was within normal after 1-month treatment with medicinal product PROTEFLAZID®. Mean AST d was decreased. Significance of t-test (less than 0.05) indicates that the mean AST in patients who received PROTEFLAZID® is significantly different from the pre-treatment mean AST starting already from the first month of treatment.

Level of total protein, concentration of α 1-globulins and concentration of α 2-globulins were within normal pre- and post-treatment with medicinal product PROTEFLAZID®. Significance of t-test (greater than 0.05) indicates that the mean of the above mentioned parameters in patients who received PROTEFLAZID® for 3 months is not significantly different from the pre-treatment mean of total protein.

Concentration of γ -globulins was within normal after 3-month treatment with medicinal product PROTEFLAZID®. However, significance of t-test (greater than 0.05) indicates that the mean of γ -globulins in patients who received PROTEFLAZID® for 3 months is not significantly different from the pre-treatment mean of γ -globulins.

Level of the circulating immune complexes was within normal pre- and post 3-month treatment with medicinal product PROTEFLAZID®. However, mean level of the circulating immune complexes was decreased. Significance of t-test (less than 0.05) indicates that the mean level of the circulating immune complexes in patients who received PROTEFLAZID® is significantly different from the pre-treatment mean level of the circulating immune complexes starting already from the first month of treatment.

Prothrombin index was within normal after 3-month treatment with medicinal product PROTEFLAZID®. Increased mean prothrombin index was observed. Significance of t-test (less than 0.05) indicates that the mean prothrombin index in patients who received PROTEFLAZID® is significantly different from the pre-treatment mean prothrombin index after the second month of treatment.

Dimensions of liver were significantly decreased after 3-month treatment with medicinal product PROTEFLAZID® based on the significance of t-test (less than 0.05).

Dimensions of spleen were not significantly decreased after 3-month treatment with medicinal product PROTEFLAZID® based on the significance of t-test (greater than 0.05). However, trend towards decrease of the spleen dimensions was observed during the treatment.

Meta-analysis of clinical studies

Patients were divided into 4 groups in the meta-analysis:

Group 1 – patients with AHB who received treatment with PROTEFLAZID® (study group) and basic therapy alone (control group);

Group 2 – patients with CHB who received treatment with PROTEFLAZID® (study group) and basic therapy alone (control group);

Group 3 – patients with AHB who received treatment with PROTEFLAZID® and plasmapheresis (study group) and basic therapy alone (control group);

Group 4 – patients with CHB who received treatment with PROTEFLAZID® and plasmapheresis (study group) and basic therapy alone (control group).

Sonographic signs of the hepatic injury

Figure 1 shows the forest plot with the results of meta-analysis based on the determination of odds ratio of rate of the increased liver echogenicity secondary to the treatment with PROTEFLAZID® during 6 months.

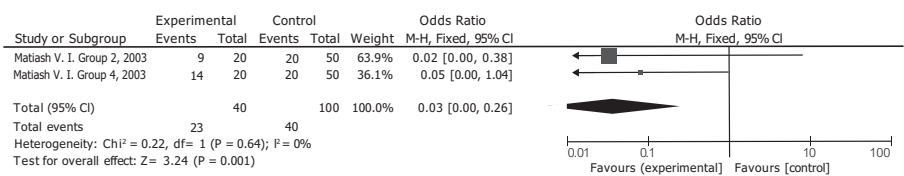


Figure 1. Results of meta-analysis based on the determination of odds ratio of rate of the increased liver echogenicity secondary to the treatment with PROTEFLAZID® during 6 months

Values of χ -squared test ($P = 0.64$) and I2-test ($I^2 = 0\%$) indicate the homogeneity of these studies; therefore, the fixed effect model is selected. P-value of Fisher test ($P = 0.001$) demonstrates the significance of the selected effect.

Value of the odds ratio ($OR = 0.03$) indicates that the probability of rate of increased liver echogenicity secondary to the treatment with medicinal product PROTEFLAZID® during 6 months is 33.3-fold lower than pre-treatment.

The largest weight has the study conducted by Matiash V. I. (2003), which indicates that the signs of the hepatic injury were observed more often in the patients from group 2 (CHB – patients who did not receive the medicinal product PROTEFLAZID®) compared with groups of patients who received medicinal product PROTEFLAZID® [11].

Figure 2 shows the forest plot with the results of meta-analysis based on the determination of odds ratio of rate of the increased liver echogenicity 3 months after the treatment with PROTEFLAZID®.

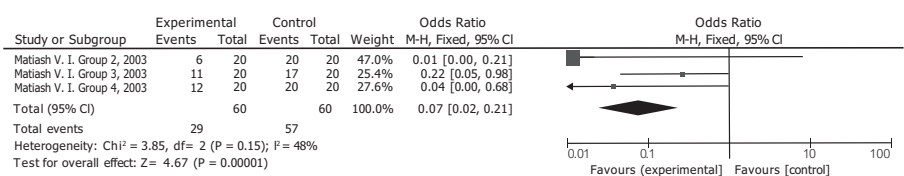


Figure 2. Results of meta-analysis based on the determination of odds ratio of rate of the increased liver echogenicity 3 months after the treatment with PROTEFLAZID®

Values of χ -squared test ($P = 0.15$) and I2-test ($I^2 = 48\%$) indicate the homogeneity of these studies; therefore, the fixed effect model is selected. Value of Fisher test ($P = 0.0001$) demonstrates the significance of the selected effect.

Value of the odds ratio (OR = 0.07) indicates that the probability of rate of increased liver echogenicity 3 months after the treatment with medicinal product PROTEFLAZID® is 14.3-fold lower than pre-treatment.

Figure 3 shows the forest plot with the results of meta-analysis based on the determination of odds ratio of rate of the fine granular liver structure after 3-month treatment with medicinal product PROTEFLAZID®.

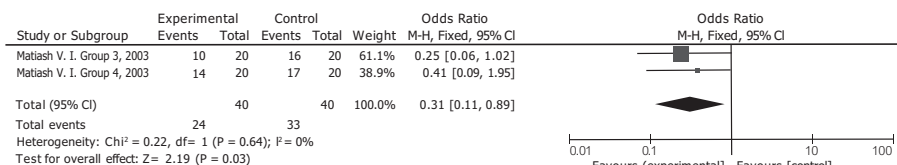


Figure 3. Results of meta-analysis based on the determination of odds ratio of rate of the fine granular liver structure 3 months after treatment with medicinal product PROTEFLAZID®

Values of χ -squared test ($P = 0.64$) and I²-test ($I^2 = 0\%$) indicate the homogeneity of these studies; therefore, the fixed effect model is selected. Value of Fisher test ($P = 0.03$) demonstrates the significance of the selected effect.

Value of the odds ratio (OR = 0.31) indicates that the probability of rate of the fine granular liver structure 3 months after treatment with medicinal product PROTEFLAZID® is 3.2-fold lower than pre-treatment.

The largest weight has the study conducted by Matias V. I. (2003), which indicates that the normalization of transferase activity parameters, absence of cholestatic syndrome, restoration of liver structure and reduction of its granularity were observed in group 3 (20 patients with AHB who received the medicinal product PROTEFLAZID® in combination with plasmapheresis) after 3-month treatment [11].

Serological markers of viral hepatitis B

Figure 4 shows the forest plot with the results of meta-analysis based on the determination of odds ratio of rate of detection of the HBV markers (Ag HBe) secondary to the treatment with PROTEFLAZID® during 1 month.

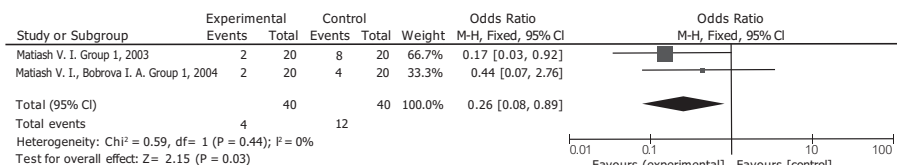


Figure 4. Results of meta-analysis based on the determination of odds ratio of rate of detection of the HBV markers (Ag HBe) secondary to the treatment with PROTEFLAZID® during 1 month

Values of χ -squared test ($P = 0.44$) and I²-test ($I^2 = 0\%$) indicate the homogeneity of these studies; therefore, the fixed effect model is selected. Value of Fisher test ($P = 0.03$) demonstrates the significance of the selected effect.

Value of the odds ratio (OR = 0.26) indicates that the probability of rate of detection of the HBV markers (Ag HBe) secondary to the treatment with PROTEFLAZID® during 1 month is 3.8-fold lower than pre-treatment.

The largest weight has the study conducted by Matiash V. I. (2003), which indicates that the positive therapeutic effect was observed in 75% of patients with AHB who received the medicinal product PROTEFLAZID® during 1 month, in particular decreased cholestatic and cytolytic syndromes and decreased hepato- and splenomegaly [11].

Figure 5 shows the forest plot with the results of meta-analysis based on the determination of odds ratio of rate of detection of the HBV markers (IgM Hbc) secondary to the treatment with medicinal product PROTEFLAZID® during 3 months.

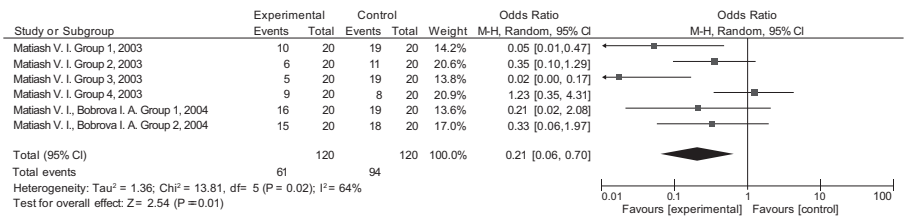


Figure 5. Results of meta-analysis based on the determination of odds ratio of rate of the detection of the HBV markers (IgM Hbc) secondary to the treatment with medicinal product PROTEFLAZID® during 3 months

Values of χ -squared test ($P = 0.02$) and I2-test ($I^2 = 64\%$) indicate the homogeneity of these studies; therefore, the fixed effect model is selected. Value of Fisher test ($P = 0.01$) demonstrates the significance of the selected effect.

Value of the odds ratio ($OR = 0.21$) indicates that the probability of rate of detection of the HBV markers (IgM Hbc) secondary to the treatment with PROTEFLAZID® during 3 months is 4.8-fold lower than pre-treatment.

The largest weight has the study conducted by Matiash V. I. (2003), which indicates that after 3-month treatment with medicinal product PROTEFLAZID®, the number of patients with antigens and blood viremia was decreased by 25% [11].

Figure 6 shows the forest plot with the results of meta-analysis based on the determination of odds ratio of rate of detection of the HBV markers (IgM Hbc) secondary to the treatment with medicinal product PROTEFLAZID® during 6 months.

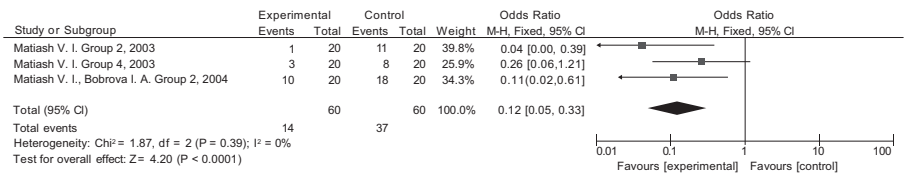


Figure 6. Results of meta-analysis based on the determination of odds ratio of rate of the detection of the HBV markers (IgM Hbc) secondary to the treatment with medicinal product PROTEFLAZID® during 6 months

Values of χ -squared test ($P = 0.39$) and I2-test ($I^2 = 0\%$) indicate the homogeneity of these studies; therefore, the fixed effect model is selected. Value of Fisher test ($P = 0.0001$) demonstrates the significance of the selected effect.

Value of the odds ratio (OR = 0.12) indicates that the probability of rate of detection of the HBV markers (IgM HbC) secondary to the treatment with PROTEFLAZID® during 6 months is 8.3-fold lower than pre-treatment.

The largest weight has the study conducted by Matiash V. I. (2003), which indicates that 6-month treatment with medicinal product PROTEFLAZID® causes the regression of the pathological process in 90% of cases and absence of antigenemia and viremia in blood in 95% of cases [11].

Figure 7 shows the forest plot with the results of meta-analysis based on the determination of odds ratio of rate of detection of HBV DNA in blood by PCR method secondary to the treatment with medicinal product PROTEFLAZID® during 3 months.

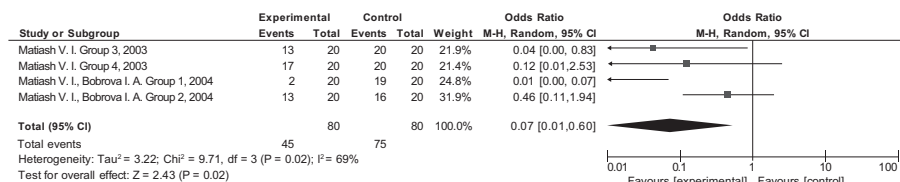


Figure 7. Results of meta-analysis based on the determination of odds ratio of rate of the detection of HBV DNA in blood by PCR method secondary to the treatment with medicinal product PROTEFLAZID® during 3 months

Values of χ -squared test (P = 0.02) and I²-test (I² = 69%) indicate the homogeneity of these studies; therefore, the fixed effect model is selected. Value of Fisher test (P = 0.02) demonstrates the significance of the selected effect.

Value of the odds ratio (OR = 0.07) indicates that the probability of rate of detection of the HBV DNA in blood by PCR method secondary to the treatment with medicinal product PROTEFLAZID® during 3 months is 14.3-fold lower than pre-treatment.

The largest weight has the study conducted by Matiash V. I. et al. (2004), which indicates that using medicinal product PROTEFLAZID® allowed decreasing the number of patients with antigenemia and viremia in blood by 25% [8].

Figure 8 shows the forest plot with the results of meta-analysis based on the determination of odds ratio of rate of detection of the HBV DNA in blood by PCR method secondary to the treatment with medicinal product PROTEFLAZID® during 6 months.

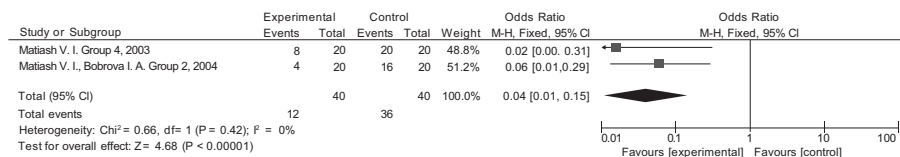


Figure 8. Results of meta-analysis based on the determination of odds ratio of rate of the detection of the HBV DNA in blood by PCR method secondary to the treatment with medicinal product PROTEFLAZID® during 6 months

Values of χ -squared test (P = 0.42) and I²-test (I² = 0%) indicate the homogeneity of these studies; therefore, the fixed effect model is selected. Value of Fisher test (P = 0.0001) demonstrates the significance of the selected effect.

Value of the odds ratio ($OR = 0.04$) indicates that the probability of rate of detection of the HBV DNA in blood by PCR method secondary to the treatment with medicinal product PROTEFLAZID® during 3 months is 25-fold lower than pre-treatment.

The largest weight has the study conducted by Matiash V. I. et al. (2004), which indicates that after 6-month treatment with medicinal product PROTEFLAZID®, the sustained positive clinical effect was observed with the normalization of blood transferases and decreased viremia and antigens in blood in 85% of patients [8].

The sensitivity analysis was performed to evaluate the effect of each individual study on the pooled data. Sensitivity analysis results showed that no individual study had the significant effect on the pooled data indicating the statistically reliable results.

Funnel plot was used to evaluate the bias of publications included to the study (Figures 9–16). Almost all values of ES in Figures 9–16 are within the funnel indicating the absence of the systematic error.

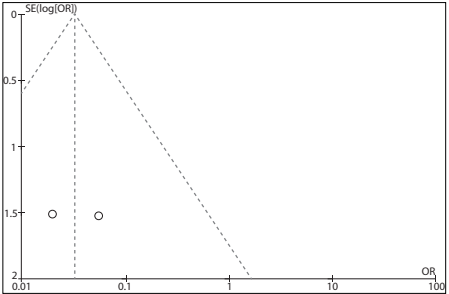


Figure 9. Funnel plot for parameter “Rate of detection of the increased liver echogenicity secondary to the treatment with medicinal product PROTEFLAZID® during 6 months”

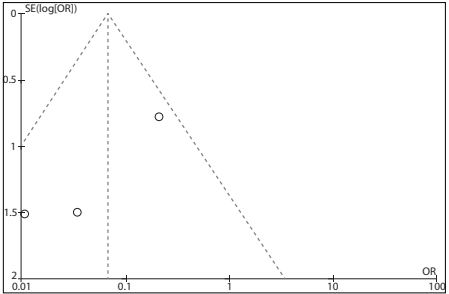


Figure 10. Funnel plot for parameter “Rate of detection of the increased liver echogenicity 3 months after the treatment with medicinal product PROTEFLAZID®”

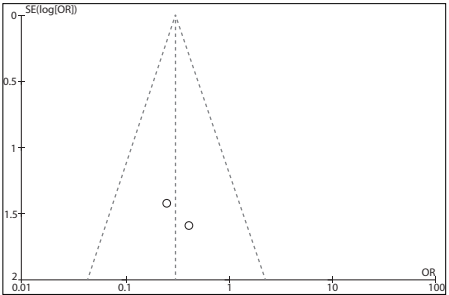


Figure 11. Funnel plot for parameter “Rate of detection of the fine granular liver structure 3 months after treatment with medicinal product PROTEFLAZID®”

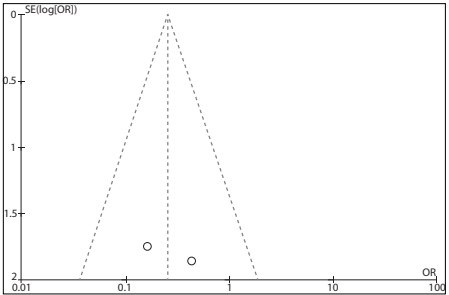


Figure 12. Funnel plot for parameter “Rate of detection of the HBV markers (Ag HBe) secondary to the treatment with medicinal product PROTEFLAZID® during 1 month”

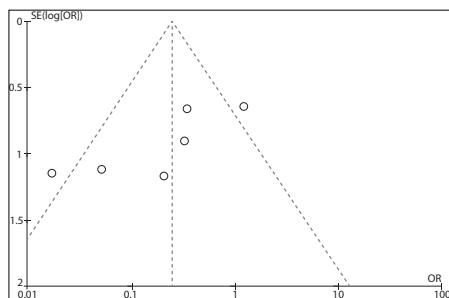


**Figure 13. Funnel plot for parameter
“Rate of detection of the HBV markers
(IgM HBc) secondary to the treatment with
medicinal product PROTEFLAZID®
during 3 months”**

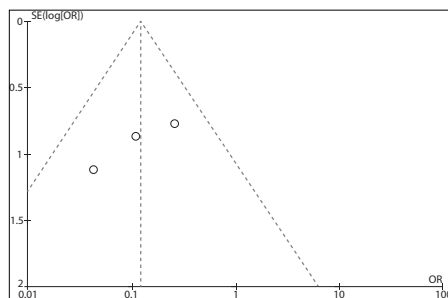


**Figure 14. Funnel plot for parameter
“Rate of detection of the HBV markers
(IgM HBc) secondary to the treatment with
medicinal product PROTEFLAZID®
during 6 months”**

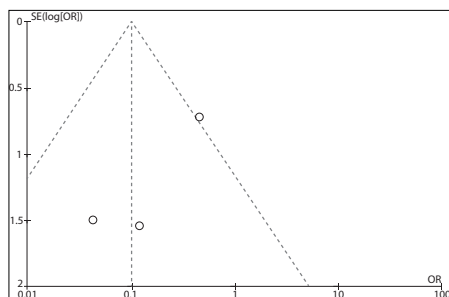


**Figure 15. Funnel plot for parameter
“Rate of detection of the HBV DNA in blood
by PCR method secondary to the treatment
with medicinal product PROTEFLAZID®
during 3 months”**

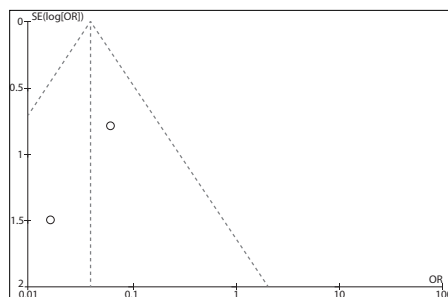


**Figure 16. Funnel plot for parameter
“Rate of detection of the HBV DNA in blood
by PCR method secondary to the treatment
with medicinal product PROTEFLAZID®
during 6 months”**

Conclusions

Statistical analysis allowed increasing the evidence base of the efficacy and safety of the medicinal product PROTEFLAZID® used for the treatment of viral hepatitis in the adult patients.

Eight (8) case-control studies involving 463 adult patients (conducted in 2000–2004) were used in the statistical analysis of the clinical studies (based on the meta-analysis and t-test) to evaluate the clinical efficacy of medicinal product PROTEFLAZID® used in the treatment of patients with the viral hepatitis (B and C).

Based on the performed statistical analyses, it is possible to state that the following findings are observed in the patients due to the therapy with medicinal product PROTEFLAZID®:

- Total bilirubin is within normal after 2-months treatment. However, decrease of the mean total bilirubin level is observed; it is statistically significant already after 1 month of treatment.

- ALT w is within normal after 2-months treatment. However, decrease of the mean ALT w level is observed; it is statistically significant already after 1 month of treatment.
- ALT d is within normal after 1-month treatment. Decrease of the mean ALT d level is observed; it is statistically significant already after 1 month of treatment.
- AST w is within normal after 1-month treatment. However, decrease of the mean AST w level is observed; it is statistically significant already after 1 month of treatment.
- AST d is within normal after 1-month treatment. However, decrease of the mean AST d level is observed; it is statistically significant already after 1 month of treatment.
- Total protein is within normal pre and post 3-month treatment. Mean value of this parameter in the adult patients who received PROTEFLAZID® for 3 months is not significantly different from the pre-treatment mean of total protein.
- α 1-globulins are within normal pre and post 3-month treatment. Mean value of this parameter in the adult patients who received PROTEFLAZID® for 3 months is not significantly different from the pre-treatment mean of total protein.
- α 2-globulins are within normal pre and post 3-month treatment. Mean value of this parameter in the adult patients who received PROTEFLAZID® for 3 months is not significantly different from the pre-treatment mean of total protein.
- γ -globulins are within normal after 3-month treatment. Mean value of this parameter in the adult patients who received PROTEFLAZID® for 3 months is not significantly different from the pre-treatment mean of total protein.
- Circulating immune complexes are within normal pre and post 3-month treatment. However, decrease of the mean value of circulating immune complexes is observed; it is statistically significant after 1 month of treatment.
- Prothrombin index is within normal after 3-month treatment. Increase of the mean value of prothrombin index is observed; it is statistically significant after 2 months of treatment.
- Liver dimensions are significantly decreased after 3 months of treatment.
- Spleen dimensions are not significantly decreased after 3 months of treatment. However, trend towards decrease of the spleen dimensions is observed.
- Probability of rate of the increased liver echogenicity (based on findings of ultrasound examinations) secondary to the treatment during 6 months is 33.3-fold lower than pre-treatment.
- Probability of rate of the increased liver echogenicity (based on findings of ultrasound examinations) 3 months after treatment is 14.3-fold lower than pre-treatment.
- Probability of rate of the fine granular liver structure (based on findings of ultrasound examinations) 3 months after treatment is 3.2-fold lower than pre-treatment.
- Probability of rate of detection of the HBV markers (Ag HBe) secondary to the treatment during 1 month is 3.8-fold lower than pre-treatment.
- Probability of rate of detection of the HBV markers (IgM HBc) secondary to the treatment during 3 months is 4.8-fold lower than pre-treatment.
- Probability of rate of detection of the HBV markers (IgM HBc) secondary to the treatment during 6 months is 8.3-fold lower than pre-treatment.
- Probability of rate of detection of the HBV DNA in blood by PCR method secondary to the treatment during 3 months is 14.3-fold lower than pre-treatment.
- Probability of rate of detection of the HBV DNA in blood by PCR method secondary to the treatment during 6 months is 25-fold lower than pre-treatment.

Thus, the meta-analysis performed using the results of the clinical studies of efficacy and safety of medicinal product PROTEFLAZID® used in the treatment of viral hepatitis (B and C) indicates that the therapy with medicinal product PROTEFLAZID® causes the regress of the cytolytic and cholestatic syndromes, restoration of protein synthesis and hemostatic functions of the liver, decrease of hepato- and splenomegaly, restoration of the liver structure, decrease of the number of antigens and intensity of viral replication in blood.

Considering the good tolerability, high safety profile and comprehensive effect on many elements in the viral hepatitis development, medicinal product PROTEFLAZID® (drops) may be recommended as the effective and safe antiviral product for the treatment of viral hepatitis (B and C) in adults.

References:

1. Михайлов В. С., Леснікова М. В., Яценко Л. А. Звіт про науково-дослідну роботу «Метааналіз результатів клінічних досліджень ефективності і безпеки застосування лікарського препарату Протефлазид® при лікуванні вірусних гепатитів» // ДС статистики України, ДП «НДІ статистичних досліджень», Київ, Україна, 2015. С. 104.
2. Крючко Т. А., Несіна І. М. Терапевтична ефективність протівірусного препарату «Флавозід» у лікуванні хронічних гепатитів вірусної етіології у дітей // Сучасна педіатрія – 2007. – №1 (14). – С. 62–65.
3. Крючко Т. А., Несіна І. М. Ефективність застосування Протефлазиду у комплексній терапії дітей з хронічним гепатитом В // Імунологія та алергологія. – 2002. – №4. – С. 38–40.
4. Дикий Б. М., Пюрік В. Ф., Остяк Р. С., Копчак О. В., Будеркевич Л. І. Застосування ПРОТЕФЛАЗИДу у комплексному лікуванні хворих на гепатит В // Матеріали науково-практичної конференції та пленуму Асоціації інфекціоністів України «Управляються інфекції» – 14–15 травня 2003. – Івано-Франківськ.
5. Нартов П. В., Волобуєва О. В. ПРОТЕФЛАЗИД® у комплексному лікуванні хворих на гострий вірусний гепатит В // Збірник матеріалів НВК «Вірусні гепатити на практиці терапевта, сімейного лікаря та інфекціоніста. Сучасні методи діагностики та терапії». – 11–12 березня 2003. – Харків.
6. Івахів О. Л., Андрійчин М. О., Вишневецька Н. Ю., Борак В. П., Китай Г. П., Надав Л. С. Клінічна ефективність ПРОТЕФЛАЗИДу та «Циклоферону» при хронічних гепатитах В та С // Матеріали науково-практичної конференції та пленуму Асоціації інфекціоністів України. – 5–6 травня 2004 року. – Тернопіль.
7. Матяш В. І., Шевчук В. Б., Токунова Т. Л., Атаманюк В. П. Терапевтична ефективність Протефлазиду при вірусних (С та В) гепатитах // Вісник Вінницького державного університету – 2002. – №6 (2). – С. 313–314.
8. Матяш В. І., Боброва І. А., Шевчук В. Б., Власик Т. Л. Оцінка терапевтичної ефективності Протефлазиду при гострому та хронічному вірусному гепатиті В // Нова медицина – 2004. – №6 (17). – С. 1–6.
9. Відкрите дослідження з вивчення ефективності та переносимості препарату ПРОТЕФЛАЗИД® виробництва ТОВ «НВК «Екофарм» у хворих на вірусні гепатити В та С (II фаза) (Звіт з клінічного дослідження). Інститут епідеміології та інфекційних хвороб ім. Л. В. Громашевського. – К., 2000. – С. 24.

10. Матяш В. І., Боброва І. А., Шевчук В. Б., Токунова Т. Л., Рибалко С. Л., Атаманюк В. П. Фармакотерапевтичні механізми ПРОТЕФЛАЗІДу при вірусних гепатитах // Проблеми епідеміології, діагностики, клініки, лікування та профілактики інфекційних хвороб – К. – С. 381–384.
11. Розробка нових методів етіопатогенетичної терапії вірусного гепатиту В, В та С, В та D (Звіт). / Інститут епідеміології та інфекційних хвороб ім. Л. В. Громашевського. – К., 2003. – С. 31.

5.2. PROTEFLAZID®: THE SPECIFIC ACTIVITY AGAINST THE HEPATITIS C VIRUS IN PRECLINICAL STUDIES; EFFICACY AND SAFETY IN THE TREATMENT OF HEPATITIS B AND C IN CLINICAL PRACTICE (SYSTEMATIC REVIEW)

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Abstract

A systematic review of the literature shows the specific activity of PROTEFLAZID® against the virus of hepatitis C in a pre-clinical study. Confirmed the safety and effectiveness of medications PROTEFLAZID® (drops) and Flavozid® (syrup), in clinical practice in the treatment of hepatitis B and C among children (175) and adults (846 patients, including 262 pregnancy cases). The results of independent research and demonstrated the effectiveness and safety of drugs PROTEFLAZID®.

Key words: PROTEFLAZID®, Flavozid®, hepatitis B and C, efficiency, safety, comparability of treatment results.

Introduction

Chronic hepatitises B and C (CHB, CHC) are one of the most essential problems of the modern medical science and practice due to their prevalence, severe consequences of the disease (such as cirrhosis and hepatocellular carcinoma), decreased quality of life and, in the significant percentage of cases, disability of patients.

At the current stage of the medical science development, treatment protocols for chronic viral hepatitises include two main areas: the first one involves using the medicinal products ensuring the activation of mechanisms that inhibit the virus replication; the second one involves the effect on the pathogenetic mechanisms of disease development and its consequences.

The modern standards of CHC treatment use the combination of pegylated interferon- α (PegIFN- α) and Ribavirin (RBV). In 2011, Telaprevir and Boceprevir were approved to be used for the treatment of infection caused by the hepatitis C virus (HCV) genotype 1. The above mentioned medicinal products are the first-generation direct-acting antiviral agents. They belong to the group of protease inhibitors and are prescribed in combination with PegIFN and RBV. Prior to the antiviral therapy initiation, all patients should have the quantitative HCV RNA test using the sensitive molecular and genetic methods (lower limit of detection: < 15 IU/ml).

Clinical guidelines of the European Association for the Study of the Liver indicate that the specific therapy should be immediately prescribed to the patients with the marked liver fibrosis and patients with the clinically apparent extrahepatic manifestations. Time of the specific therapy initiation in patients without or with the mild fibrosis is disputable; it may be delayed till the new treatment methods are developed [1].

The antiviral therapy involves prescribing the direct- or indirect-acting antiviral agents. Specific direct antiviral activity is the property of the medicinal product to inhibit the reproduction of certain viruses. Indirect antiviral activity involves enhancing the immune system by the medicinal product. This specific review discusses the hepatitis B (DNA virus: Orthohepadnavirus of Hepadnaviridae family) and hepatitis C (RNA virus: Flavivirus genus of Flaviviridae family) viruses.

The search for the new therapeutic strategies aimed at increasing efficacy, pan-genotypic activity, reducing treatment duration, simplifying medicinal product administration, increasing tolerability and adherence to treatment is ongoing.

The antiviral products – PROTEFLAZID® (drops) and Flavozid® (syrup) manufactured by SMC “Ecopharm” LTD, Ukraine – containing the flavonoids of PROTEFLAZID® extract as active substance meet the high therapeutic requirements [2, 3].

The extract is obtained using the technology of alcoholic extraction (96% ethanol) from the herbal raw materials (wild grasses: *Calamagrostis epigeios* L. and *Deschampsia caespitosa* L.). The medicinal product contains the flavonoids (flavone and flavonol glycosides) derived from grasses presented as the stable molecular combinations of tricine, apigenin, luteolin and quercetin [3].

Study objective

Show the specific activity of medicinal product PROTEFLAZID® against HCV in pre-clinical research setting based on the available scientific data. Analyze the independent clinical observations of efficacy and safety of medicinal products PROTEFLAZID® (drops) and Flavozid® (syrup) used in the clinical settings in treatment of hepatitis B and C.

Materials and methods

Scientific publications of the preclinical and clinical studies; systematic analysis.

Results and discussion

Rybalko S. L. (2004) studied the activity of medicinal product PROTEFLAZID® against HCV in in vitro preclinical studies using experimental model of HCV infection on the cells of the rat trigeminal ganglion neurinoma (RTGN). The following values were obtained using PCR method: maximum tolerable concentration of medicinal product (for cell culture): $19.5 \mu\text{g/ml}$; minimum active concentration of medicinal product (for HCV): $0.01 \mu\text{g/ml}$; chemotherapeutical index (CTI) of medicinal product: 1950. The obtained values allowed the investigator to classify the medicinal product

PROTEFLAZID® as the highly effective direct-acting antiviral product with activity against hepatitis C virus [4, 5].

Later, the antiviral activity of medicinal product PROTEFLAZID® was confirmed in the viral-cell model of the surrogate HCV infection using the bovine viral diarrhea virus (BVDV), which like HCV belongs to Flaviviridae family. The study was conducted using MT-4 (cells of lymphoblastoid origin) cell line. The undiluted blood plasma containing different concentrations of HCV RNA and obtained from patients with hepatitis C was used as HCV-containing material. During the studies, the productive cultures of MT-4 cells transfected with HCV complementary DNA (cDNA) were obtained ensuring the stable production of hepatitis C virus. It was demonstrated that the medicinal product inhibited HCV reproduction at blood concentration of 0.37 µg/ml. The maximum tolerable concentration of medicinal product was 825 µg/ml and minimum active concentration was 0.37 µg/ml with the chemotherapeutical index equal to 2 230 [4, 5].

Similar data were received in the studies conducted by Starosyla D. B. (2014). To study the activity of medicinal product PROTEFLAZID® against HCV, the surrogate virus of hepatitis C (BVDV) and MT-4 cell culture transfected with HCV DNA were used. It was demonstrated that medicinal product PROTEFLAZID® (used at 0.375 µg/ml) effectively inhibited expression of HCV RNA and reproduction of surrogate BVDV [3].

The studies conducted by Porva Yu. I. (2010) investigated the activity of medicinal product PROTEFLAZID® in the mixed infection model. For this purpose, RTGN cell culture pretreated with the different doses of medicinal product PROTEFLAZID® was infected with HIV and then with HCV. The viral load was determined by PCR method. It was noted that HCV viral load was significantly increased (from 2 450 to 4 062 gE) in HIV + HCV mixed infection. It was also found that PROTEFLAZID® used at 2 µg/ml, 1 µg/ml, 0.2 µg/ml significantly (100%, 88%, 100%) inhibited the HCV reproduction in the mono- and mixed (HIV + HCV) infection model [5].

It should be mentioned that the spectrum of the direct antiviral action of medicinal product PROTEFLAZID® extends to other viral infections, in particular, caused by type 1 and 2 herpes virus, influenza virus, Epstein-Barr virus, HIV, cytomegalovirus, papillomavirus (types 1, 16, 31, 35, 39, 59) and adenovirus (serotype 5) [3, 6, 7].

Data obtained by Palchykivska L. H. et al. (2013) confirm that the viral enzymes – transcriptase and protease are the target of flavonoids. They demonstrated the high inhibiting activity of active substance of medicinal product PROTEFLAZID® towards in vitro viral RNA synthesis in the model transcription system of bacteriophage T7 (RNAP T7). PROTEFLAZID® is the active inhibitor of RNAP T7 transcriptional complex with IC₅₀ (50% inhibitory concentration) equal to 0.07 µg/ml. Also they provided the data pertinent to effect of PROTEFLAZID® extract on the DNA fragments in PCR settings (as the model system for screening and selection of agents that impair or block DNA synthesis). Like in inhibition of the RNA synthesis, the concentration-dependent inhibition of the amplification process is observed: IC₉₀ (at which the active substance completely inhibits DNA synthesis) is 8 µg/ml. To clarify the site at which medicinal product PROTEFLAZID® inhibits the DNA and RNA virus synthesis, molecular docking of process of fixing the extract components to the molecular model of the RNAP T7 transcription complex was performed. The authors showed that the connection is maintained due to the strong coordination bonds developed between oxygen

atoms and magnesium ion with the amino acid residue, tyrosine 639 of the transcription complex [6].

PROTEFLAZID® demonstrates the effective antiviral effect against HCV at the low level of toxicity, as evidenced by the high values of CTI parameter – 680. It should be mentioned that the level of RNA synthesis inhibition by medicinal product PROTEFLAZID® is very close to the values of efficacy of virus reproduction inhibition in vitro [6].

One of the mechanisms of antiviral effect of medicinal product PROTEFLAZID® is the interferonogenic activity. Data obtained by Zavelevych M. P. et al. (2003) indicate that the medicinal product in vitro dose-dependently stimulates the interferon (IFN) production in human WBC and in culture of subinoculated MDBK cells. Typing indicates that the medicinal product is the inducer of α - and γ -IFN [8]. The conducted studies confirmed that PROTEFLAZID® may induce IFN both in vitro and in vivo thus having the indirect effect on viruses [2, 3, 7].

In addition to the antiviral activity, the active substance of medicinal product PROTEFLAZID® also exerts the antioxidant properties: it increases cell resistance to the free-radical stress in the infections, reduces the negative effects of chemotherapy and promotes the body's adaptation to the adverse environmental conditions. The in vitro experiment demonstrated that the medicinal product inhibits the intensity of free-radical processes induced by hydrogen peroxide by more than 2 times. It was shown at the cell level that PROTEFLAZID® inhibits the generation of superoxide radical anion almost to zero within 24 hours of using the medicinal product, i.e. it maintains the antioxidant status of cells [2].

Medicinal product PROTEFLAZID® also exerts the apoptosis-modulating activity – it contributes to the primary prevention of cancer secondary to the chronic (latent) viral infections. The active substance of PROTEFLAZID® extract exerts the apoptosis-inducing effect due to the activation of the initiator caspase-9: it restores the ability of the virus-infected cells towards apoptosis reducing the activity of proliferative processes in the mutated cells [7].

Based on the acute toxicity levels, the herbal antivirals containing flavonoids – PROTEFLAZID® (drops) and Flavozid® (syrup) belong to class 5 of safe substances. The pre-clinical studies did not demonstrate the teratogenic, mutagenic, embryotoxic, cytotoxic and carcinogenic effects [2, 3, 7].

In the clinical practice, the medicinal products containing PROTEFLAZID® extract: PROTEFLAZID® (drops) and Flavozid® (syrup) are used for the treatment of hepatitis B and C. Both dosage forms may be used in adults and in children, but it is recommended to use the pediatric dosage form – Flavozid® syrup for the treatment of viral hepatitis in children under 12 years.

Efficacy and safety of medicinal product PROTEFLAZID® (drops) used in adults

At the pre-approval stage of studying the medicinal product PROTEFLAZID® (drops) in the Department of Intensive Therapy and Detoxication of SI “L.V. Hromashevskiy Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine” under the guidance of V. I. Matiash, doctor of medical sciences (2000), the controlled (by the protocol previously approved by the Pharmacological Committee of the Ministry of Health of Ukraine), open, clinical study to investigate the efficacy and tolerability of medicinal product PROTEFLAZID® (drops) was conducted in 30 patients with hepatitis B and C. The medicinal product was prescribed by the following regimen: Week 1 – 5 drops three times daily; Weeks 2 and 3 – 10 drops three times daily; Week 4 – 8 drops three times daily for 4 months without interruption.

All subjects of the study were prescribed nutritional therapy (diet No. 5). The hepatoprotectors, spasmolytics, upper gastrointestinal motility regulators, sorbents, diuretics, detoxification and other symptomatic agents were prescribed by indications. The medicinal products with the antiviral, antimetabolic, immunosuppressive or immunomodulatory effects were not prescribed during the study. It was demonstrated that the signs of the impaired overall health, on average, were resolved 7.1 days earlier in the patients who received the medicinal product PROTEFLAZID® (drops) compared with the control group and nausea and dyspeptic disorders – 4.8 days earlier. Such patients showed more significant decrease of the density and dimensions of the affected liver at the end of the treatment period (in 70% of patients who received the medicinal product versus 59.9% of patients from the control group). The positive result of the hepatic function restoration is demonstrated, in particular, by the fact that aminotransferase activity (ALT and AST) in the patients who received the medicinal product PROTEFLAZID® (drops) decreased on average by 80% until the end of the treatment period and in the control group – by 60% indicating the decreased cytolytic component that is the indicator of the chronicity process in hepatitis. At the same time, positive effect of the medicinal product PROTEFLAZID® (drops) on the restoration of protein synthesis function of the liver was noted; it was demonstrated by the rapidly increased blood albumin levels in patients receiving this medicinal product [9].

Based on the analysis of experience of using PROTEFLAZID® (drops) in 45 patients with the acute and chronic hepatitis B and C, Matiash V. I. et al. (2002) made a conclusion that the product is the etiopathogenetic agent for the treatment of hepatitis B and C, in particular their acute forms (basic symptomatic therapy included plasmapheresis, glucose and saline solutions, amino acids). The medicinal product has positive effect on the restoration of the structural and functional condition of the affected liver, its homeostatic functions (detoxification, excretory, protein synthesis), it causes the regression of the clinical syndromes (cholestatic, cytolytic, dyspeptic, asthenic).

Later, Matiash V. I. (2003) conducted the research study “Development of new methods of etiopathogenetic therapy of viral hepatitis B and C” where they also used PROTEFLAZID® (drops) and its combination with the efferent treatment method – discrete plasmapheresis secondary to the symptomatic therapy to treat 60 patients with the acute and chronic hepatitis B, B + C (20 patients) and B + D (5 patients). Based on the analysis of the obtained results, it was found out that provided the continuous administration of the medicinal product for 3 months, regression of the pathological process with restoration of the liver structure was observed in 80% of patients with the complicated acute hepatitis B and in 70% of patients with chronic hepatitis B who received PROTEFLAZID® (drops) for 6 months [12]. Co-infection with hepatitis B and D viruses (HDV) is more severe than infection with only one virus. As HDV is a satellite of HBV, i.e. able for reproduction only in the HBV-infected cells, the indirect effect of medicinal product PROTEFLAZID® on HDV may be assumed by inhibiting HBV reproduction.

Summarizing the experience of using PROTEFLAZID® (drops) in the combination therapy of the complicated acute and chronic hepatitis B and B + C, Matiash V. I. et al. (2004) confirmed that the medicinal product used secondary to the pathogenetic therapy (glucose and saline solutions, vitamins, Glutargin, vasoactive agents and in the most difficult cases – Refortan, plasmapheresis) has the positive effect on the regression of the disease clinical signs and kinetics of restoration of the homeostatic hepatic functions (detoxification,

excretory, protein synthesis), alleviates the cholestatic and cytolytic syndromes ensuring the more effective decrease of bilirubin levels and transaminase activity. It is stated that PROTEFLAZID® (drops) is well tolerated by the patients; it does not cause the adverse side effects both in the acute and chronic hepatitis and may be successfully used in patients with the concomitant pathology, particularly if there are contraindications to the use of interferon products [13].

From a scientific point of view, co-administration of the exogenous interferon and medicinal product PROTEFLAZID® (drops) is justified in the treatment of the viral hepatitis, as this product, in addition to the antiviral effect, is capable to induce the synthesis of endogenous α - and γ -IFN. Efficacy of such approach is shown in the article of Ivakhiv A. L. et al. (2004). The study conducted in Ternopil Medical Academy named after I. Ya. Horbachevskii evaluated the efficacy of the low molecular weight inducer of endogenous IFN cycloferon and its combination with medicinal product PROTEFLAZID® (drops) in the combination therapy of patients with CHB and CHC. It was found out that biochemical remission occurred faster in the patients with CHC who received PROTEFLAZID® (drops) and cycloferon compared with the patients who received cycloferon alone. In particular, when they were discharged from the hospital, their serum bilirubin level was 1.5 times lower. Changes of the biochemical parameters in the patients with CHB were even more pronounced over time: ALT activity decreased up to 0.9 mmol/h · l versus 1.4 mmol/h · l in the reference group. Duration of inpatient treatment was decreased (16.7 and 28.5 inpatient days, respectively). The authors emphasize that the concomitant administration of the concentrated exogenous interferon products and interferon inducers (to which PROTEFLAZID® (drops) belongs) is a promising trend to improve the therapy of chronic hepatitis B and C [14].

In general, similar data on the efficacy and safety of the medicinal product PROTEFLAZID® (drops) were obtained by the employees of Kharkiv Medical Academy of Postgraduate Education (Nartov P. V., Volobuiev O. V., 2003) who use the medicinal product in addition to the standard basic therapy during the treatment of 35 patients with the acute hepatitis B. Most notably, decreased severity of the intoxication syndrome, improved appetite, resolved general weakness and nausea were observed in those patients. The 1-month follow-up monitoring of those patients demonstrated the normalized ALT activity on 80% of patients and undetectable blood HBV by PCR method in 82% [15].

Dykyi B. M. et al. (2003) conducted the study of medicinal product PROTEFLAZID® (drops) in Ivano-Frankivsk Medical Academy. The study involved 17 patients (study group) who received PROTEFLAZID® secondary to the conventional therapy and 20 patients (control group) who received the conventional therapy alone. It was demonstrated that after 1-week administration of the medicinal product PROTEFLAZID®, the manifestations of asthenic-vegetative, dyspeptic and icteric syndromes were decreased in patients with the prolonged course of hepatitis B and after 2-week therapy, the manifestations of cytolytic, mesenchymal-inflammatory syndromes and signs of cholestasis were significantly reduced. The complete normalization of the biochemistry parameters was observed in 84.4% of patients versus 68.7% from the control group [16].

It should be mentioned that information is available about the efficacious use of the medicinal product PROTEFLAZID® in treatment of hepatitis A virus (HAV) infection. In particular, Saveliev V. H., Bondarieva V. V. (2005) conducted the study at the site of Zaporizhzhia Medical University; the study involved 65 patients with the viral hepatitises of different

etiology – 30 patients had HAV and 6 patients had HAV secondary to CHC. It was demonstrated that administration of medicinal product PROTEFLAZID® secondary to the basic therapy of the acute HAV caused more rapid relief of intoxication symptoms, decreased duration of jaundice and improved general well-being in 83.4% of patients. The decrease of the cytolytic and cholestatic syndromes was observed in 76.8%. Positive changes of the bilirubin level were noted in 79.6% of patients; the improvement of the intestinal function and rapid relief of dyspeptic syndrome were observed in 52% of patients. Patients with HAV secondary to CHC (18%) and patients with CHC who received treatment with the medicinal product demonstrated the short-term exacerbation of cytolytic syndrome for 5–7 days with further positive changes of the biochemical parameters and decreased liver dimensions (based on the ultrasound findings) [17].

Efficacy and safety of medicinal products PROTEFLAZID® (drops) and Flavozid® (syrup) used in children

Major part of the available publications is devoted to the analysis of the results of using medicinal products PROTEFLAZID® (drops) and Flavozid® (syrup) in the treatment of hepatitis B and C in children. Experience of using medicinal product PROTEFLAZID® (drops) in the treatment of children with hepatitis B and C was analyzed at the Chair of Hospital Pediatrics and Pediatric Infectious Diseases of the State Higher Education Institution “Ukrainian Medical and Dental Academy” (the city of Poltava). In particular, Kriuchko T. A., Nesyna I. M. (2002) conducted the study of efficacy of medicinal product PROTEFLAZID® used in the combination therapy in children with the chronic hepatitis B and noted the regression of the clinical symptoms, normalization of the biochemical parameters, decrease of the parenchymal inflammatory events, restoration of the physiological hepatic structure within a short time in the group of children who received the medicinal product compared with the control group of children [18]. The further studies demonstrated more rapid viral and biochemical remission and significantly increased concentration of α - and γ -IFN (which is the indicator of stimulation of patient’s defense mechanisms) in the children who received PROTEFLAZID® (drops) [19–21].

Abdalaal Mohammed M. A. (2006) demonstrated the efficacy of the combination therapy with addition of PROTEFLAZID® (drops), membrane stabilizer thiotriazoline to the basic etiopathogenetic therapy (Enterogel, Hepabene, Galstena, diet No. 5) in children with the chronic hepatitis C. Primary virological remission, normalization of the clinical and laboratory parameters, stabilization of the fibroplastic changes and correction of hepatic hemodynamic disorders were observed in 57.1% of patients who received the medicinal product. This article showed not only its positive effect on the changes of disease clinical symptoms over time, but, what is especially significant, the improved hepatic blood flow and significantly increased level of IFN, especially α -IFN, after the course of treatment was completed indicating the inhibition of the HCV replication process [22, 23].

The controlled (according to the protocol previously approved by the State Expert Center of the Ministry of Health of Ukraine) open study to evaluate the efficacy and tolerability of medicinal product Flavozid® (syrup) used in children with CHB та CHC was conducted under the guidance of professor Kriuchko T. A (2008); the study involved 71 patients aged 3–16 years. Flavozid® (syrup) is prescribed to the pediatric patients from birth.

The medicinal product was prescribed in accordance with the recommendations and age-specific doses secondary to the basic therapy that included the pathogenetic and

symptomatic agents, hepatoprotectors, antioxidants, intestinal sorbents and medicinal products that improve micro- and lymphatic circulation. The replicative phase of the infectious process was detected in 27.7% of patients and integrative phase – in 72.3%. The integral assessment of the clinical efficacy performed by determining the paired difference between clinical indices 3 months pre- and post-treatment demonstrated that it was 6.52 and after 6 months – 8.74 with $p < 0.05$, which indicates the high efficacy of the medicinal product. It is also demonstrated by the results of analysis of the biochemical parameter changes that reflect the functional hepatic condition; in particular, decreased levels of the serum total, direct and indirect bilirubin, ALT and AST were observed. The authors also obtained the data about the higher levels of endogenous α - and γ -IFN in the patients who received the medicinal product Flavozid® (syrup) indicating that more rapid improvement in the patients was associated with the property of this product to stimulate the synthesis of these interferons. In particular, level of α -INF was increased by more than 3-fold at the end of the treatment in the patients who received therapy involving the medicinal product Flavozid®, while that level was only 1.5-fold increased in the patients who received basic therapy alone. In general, the same pattern was observed in the analysis of γ -IFN levels and viral load in the patients from the different groups. During the study, no objective or subjective manifestations of adverse reactions were observed in the children from different age groups when they received medicinal product Flavozid® (syrup) [24, 25].

Efficacy and safety of medicinal product PROTEFLAZID® (drops) used in pregnant

Hepatitis B is the real threat to the life of a pregnant woman, fetus and newborn. Deterioration in the second half of pregnancy can be complicated by the acute hepatic failure with encephalopathy and coma with high mortality rate (the mortality rate outside of pregnancy is 0.4–2%, in pregnant women – 3 times higher). HCV effect on the gestation process has not been detected; however, HCV-positive women had a higher rate of preterm births, with some studies indicating the increase of up to 29% (overall rate: 6–10% of all births). Treatment of viral hepatitis during the pregnancy is associated with certain difficulties, primarily due to the existing restrictions in the use of certain medicinal products during this period. Data on the high efficacy and safety of the medicinal product PROTEFLAZID® (drops) obtained during the treatment of the acute and chronic hepatitis B and C became the basis for using this product during the pregnancy. The medicinal product is not contraindicated for using in pregnant women, as it does not have teratogenic, mutagenic and embryotoxic effects; therefore, the use of the antiviral and immunomodulatory properties of medicinal product PROTEFLAZID® may ensure the decreased rate of the perinatal complications [26].

Mitsoda R. M. (2005–2007) analyzed the experience of using the medicinal product PROTEFLAZID® (drops) in pregnant women with hepatitis B and C, in particular in the patients with the active replication of pathogens. To correct the impairments caused by HBV and HCV, the medicinal product was used (as it does not have teratogenic effect) in addition to the basic therapy (Glutargin, Duphalac, Enterosgel, Ascorutin) in more than 100 women. The rate of pathological blood loss during childbirth was almost 3-fold lower in the group of women who received PROTEFLAZID®; rate of anemia of pregnancy was lower; rate of preterm births was 2.2-fold lower and the inflammatory complications were much less common in the postpartum period. This fact is explained by the immunomodulatory properties of medicinal product PROTEFLAZID® (drops): both systemic and local immunity is improved

secondary to using the product. It results in the improved ability to resist the infectious agents in the postpartum period [27–29].

Obtained positive experience of using medicinal product PROTEFLAZID® (drops) was the basis to include it to the regimens of prevention and treatment of the obstetric complications in viral hepatitis.

On the other hand, according to Mitsoda R. M. (2006), prescription of the medicinal product PROTEFLAZID® (drops) to the women with HBV or HCV replicative activity during gestation decreased the total rate of asphyxia, hyporeflexia and acute ischemic injury of the central nervous system in the early neonatal period of the fetus [30].

The specialists from the Research Institute of Obstetrics and Gynecology in Uzbekistan received similar data confirming the efficacy and safety of medicinal product PROTEFLAZID® (drops) used in pregnant women and patients with the acute and chronic hepatitis B. In particular, Zarzhova Z. Sh. (2008) showed that administration of the medicinal product in pregnant women allowed reducing the birth rate of children with pathology by 2 times, improving the adaptation period and decreasing the risk of HBV transmitting to children [31]. On the other hand, Zaripovyi Z. Sh. et al. (2008) demonstrated that administration of the medicinal product PROTEFLAZID® (drops) by the pregnant women with the chronic hepatitis B leads to the improved blood circulation and decreased dystrophic changes in both the maternal and fetal parts of the placenta. The morphological examination demonstrated the dilated vessels and resolution of edema events in the placental tissue stroma, which reduces the incidence of the obstetric and perinatal pathology [32].

The specialists from P. L. Shupyk National Medical Academy of Postgraduate Education studied the problems of the gestational process in the women with a history of hepatitis B. Pekhno N. V. (2005), like the above mentioned authors, emphasizes that the placental insufficiency is one of the leading reasons explaining the high rates of perinatal fetal pathology in pregnant women with the hepatitis B in the history. It was demonstrated that addition of medicinal product PROTEFLAZID® (drops) to the generally accepted combination of the therapeutic and preventive measures for the placental insufficiency correction had the positive effect on the microcirculation condition in the pregnant women. The improved condition of the microcirculatory vessels during the pregnancy (decreased rate of pathological abnormalities in all three levels – vascular, intravascular and perivascular) and improved pregnancy course and childbirth were noted; it was evidenced by the decreased risk of the threatened miscarriage (from 22% in women who did not receive the medicinal product to 10% in the group who received PROTEFLAZID®), anemia (from 38% to 18%), premature rupture of the fetal membranes (from 46% to 20%) and intrauterine growth retardation (from 40% to 22%). Administration of the medicinal product also allowed avoiding development of the decompensated placental insufficiency with the formation of compensated placental insufficiency in 86% of cases and subcompensated placental insufficiency in 14% of cases and reducing the perinatal fetal pathology rate in pregnant women infected with HBV by half (from 40% to 20%) [33].

Summarizing the above mentioned, it should be noted that the data obtained in the clinical practice are consistent with the results of preclinical studies and confirm the specific activity of the medicinal product PROTEFLAZID® against hepatitis B and C.

The main results of 23 clinical studies are provided in Table below.

Table. Results of clinical studies of efficacy and safety of the medicinal products PROTEFLAZID® (drops) and Flavozid® (syrup) used in treatment hepatitis B and C in the children, pregnant and adults in the period from 2000

№	Author, year, source	Number of patients: total in study	Efficacy of using medicinal products PROTEFLAZID® / Flavozid®
Treatment of hepatitis B and C in adults			
1	Matiash V. I., 2000 [9]	45/30	Bilirubin level was actively decreased from 293.7 ± 6.7 to 43.8 ± 4.5 $\mu\text{mol/l}$ secondary to the administration of medicinal product PROTEFLAZID®; in control group (basic therapy) – from 278.8 ± 7.3 to 66.7 ± 3.9 $\mu\text{mol/l}$ ($p < 0.05$). In study group, the bilirubin level was normalized, on average, 8.2 ± 0.4 days earlier than in control group; ALT activity was decreased more intensively ($p < 0.05$) up to Week 4 – almost by 1.5 ± 0.2 times (from 22.6 ± 1.4 to 3.2 ± 0.5 mmol/h – study group, from 21.1 ± 1.5 to 4.9 ± 0.6 mmol/h – control group). Blood albumin level increased from $47.1 \pm 0.7\%$ to $52.9 \pm 0.5\%$ – study group, from 47.6 ± 0.6 to $49.1 \pm 0.7\%$ – control group. Hepatic fibrosis elements in the study group were observed half as often as in the control group; liver dimensions in the study group were decreased by 2.8 ± 0.2 cm by 4–6 weeks earlier.
2	Matiash V. I. et al., 2002 [10]	45/25	One-month administration of PROTEFLAZID® resulted in the decrease of the asthenic, vegetative and dyspeptic events in 60.0% of patients (in control group [basic therapy] – in 45.0%). The recovery of the satisfactory condition was observed, on average, 7.1 days earlier than in the control group. The hyperbilirubinemia levels at the end of Week 4 were decreased in 20.0% of patients (control group: 15.0%), on average by 6.8 times (control group: 4.2 times). At treatment month 2, ALT level was decreased almost by 80% in the study group and only by 60% in the control group (1.5-fold). Decrease of dysproteinemia, decrease of gamma fraction levels from 22.3 to 19.0% (control group: from 21.9 to 20.3%), increase of albumin level – up to 52.7% (control group: up to 49.1%). Events of chronicity and hepatic parenchymal fibrous degeneration were observed in 20.0% of patients in the study group and in 30.0% of the control group. Reduction of liver borders (by percussion) by 2.8 cm was observed 6.9 days earlier in the study group.

3	Matiash V. I. et al., 2002 [11]	85/85	Blood bilirubin levels secondary to 1-month treatment with the medicinal product PROTEFLAZID® in the acute HBV were normalized in 86.0% of patients, ALT activity – in 82.0%. Blood protein spectrum was normalized: Decreased dysproteinemia, decreased globulin content and, in particular, its gamma fraction, from 22.3 ± 0.3 to $19.0 \pm 0.2\%$ in the chronic disease, from 21.9 ± 0.2 to $20.3 \pm 0.3\%$ – in the acute disease and, respectively, increased albumin level – from 49.1% to 52.7% was observed in 76.0% of patients during the second month – 90.4%. Decrease of liver dimensions (by 2.8 ± 0.2 cm) and its density during the first month was observed in 98.8%. Moderate hepatic parenchymal fibrous degeneration was observed only in 28.0% of patients with the acute disease. Decrease of the asthenic, vegetative and dyspeptic events of gastrointestinal dyskinesia was observed in 91.6% of patients within 2–4 weeks.
4	Dykyi B. M., 2003 [16]	37/17	After 1-week administration of the medicinal product PROTEFLAZID®, patients from the study group showed the decreased asthenic-vegetative, pain, dyspeptic and icteric syndromes; decreased liver dimensions and density were recorded later (in control group [basic therapy] – 10–12 days later). After 2-week treatment, significantly decreased parameters of the cytolytic, mesenchymal-inflammatory syndromes and manifestations of cholestasis were observed: ALT and AST activity was decreased by 1.5 times, result of thymol test – by 20–24%, blood gamma content – by 19–23%, total bilirubin – by 1.4 times; content of diene conjugate and malondialdehyde was significantly lower than in patients from the control group, respectively, 2.46 ± 0.18 mmol/l and 19.26 ± 0.56 mmol/l versus 2.98 ± 0.22 mmol/l and 21.79 ± 0.74 mmol/l. Complete normalization of the biochemistry parameters in the study group was observed in 84.4% of patients and in control group – in 68.7%.
5	Matiash V. I., 2003 [12]	120/80	Secondary to 3-month administration of the medicinal product PROTEFLAZID®, regression of the pathological process in the complicated acute HBV was observed in 80.0% of patients and in 100% of patients who received the combination of PROTEFLAZID® and plasmapheresis. The regression of the pathological process in the chronic HBV was observed in 70.0% of patients who received the medicinal product for 6 months and in 90.0% of patients who received the combination of PROTEFLAZID® and plasmapheresis. The sustained therapeutic effect (as evidenced by normalization of transferase activity, absence of antigenemia and viremia) in the chronic HBV was observed in 55.0% of patients who received the medicinal product alone and in 75.0% of patients who received the combination of PROTEFLAZID® and plasmapheresis.

6	Nartov P. V., Volobuiev O. V., 2003 [15]	35/5	Patients who received the medicinal product PROTEFLAZID® required less time to improve the health condition compared with the control group (basic therapy) – general weakness, nausea were resolved, appetite was improved, liver dimensions were normalized, skin jaundice was decreased. Change of the direct and free bilirubin levels occurred much earlier in the patients who received the medicinal product. In particular, prior to the discharge from the hospital, total bilirubin level was 27.1 ± 2.0 mmol/l and in the patients from the control group – 48.6 ± 1.3 mmol/l. Duration of the hospital stay was reduced: in the mild disease: 22.4 ± 0.4 (control group: 27.9 ± 2.2), in the moderate disease: 28.1 ± 1.9 (control group: 36.1 ± 3.3).
7	Ivakhiv O. L., 2004 [14]	56/30	PROTEFLAZID® used alone and in combination therapy with Cycloferon promoted the resolution of the clinical manifestations of the disease (nausea, poor appetite, general weakness) and normalized the liver dimensions. Biochemical remission in CHC patients occurred faster: when the patients were discharged from the hospital, serum bilirubin was significantly lower – $25.8 \mu\text{mol/l}$ versus $36.6 \mu\text{mol/l}$ in the control group (basic therapy); more significant trend towards ALT activity normalization was observed – 1.3 mmol/l and 1.5 mmol/l/hour, respectively. In the patients with CHB, the positive changes of the biochemistry parameters were even more marked: serum bilirubin at discharge was 24.6 mmol/l versus $34.8 \mu\text{mol/l}$ in patients from the control group; ALT activity was significantly lower – $0.9 \text{ mmol/h} \cdot \text{l}$ versus $1.4 \text{ mmol/h} \cdot \text{l}$, respectively. Duration of hospital stay was reduced – mean number of inpatient days was 16.7 ± 1.4 versus 28.5 ± 2.6 in the patients from the control group.

8	Matiashev V. I., 2004 [13]	40/20	Acute hepatitis B. One-month administration of PROTEFLAZID®: it decreased the severity of the cholestatic and cytolytic syndromes, in particular, transferase activity – by 43.7%, total bilirubin level – by 65.3%, reduced the liver dimensions by 32.3%. Improved general health condition was observed in 88% of patients. Three-month administration: transferase activity parameters were normalized in 80% of patients and cholestatic syndrome was absent; hepato- and splenomegaly were decreased by 62%, hepatic structure was restored; number of patients with the antigens and blood viremia decreased by 25%. CHB. One-month administration: decreased severity of the cholestatic and cytolytic syndromes, in particular, transferase activity – by 37.8%, total bilirubin level – more than 80% up to the normal level in 2/3 of patients; decreased hepato- and splenomegaly – by 23.8%; moderate improvement of the general health condition, particularly function of the gastrointestinal tract; decreased severity of the asthenic-vegetative syndrome. Six-month administration: decreased severity of the cytolytic syndrome and absence of the cholestatic syndrome in 85% of patients, particularly decreased blood level of circulating immune complexes in 65% of patients; sustained improvement of the general health condition: transferase activity was decreased by 81.5% (with normalization in 55% of patients); hepato- and splenomegaly were decreased by 65%, hepatic structure was restored.
Treatment of hepatitis B and C in children			
9	Kriuchko T. A., Nesyna I. M., 2002 [18]	18/12	PROTEFLAZID® improved the clinical picture during 11.3 ± 1.4 days, in control group (basic therapy) – 16.2 ± 1.7 days. The mean duration of hyperbilirubinemia in children from group 1 was 12.6 ± 1.12 days, in control group – 18.3 ± 2.12 days. Simultaneously with the positive changes of the laboratory findings, the significant decrease of the liver and spleen dimensions, resolution of the dyspeptic events were observed starting from Week 4 of administration of medicinal product.
10	Kriuchko T. A., Nesyna I. M., Mohammed M. A. Abdalaal, 2005 [19]	57/27	Secondary to administration of medicinal product PROTEFLAZID®, even in the virus replication phase and in the moderate hyperenzymemia, symptoms of intoxication (increased fatigue, decreased appetite and nausea) were observed only in 11.1% of patients and vascular extrahepatic signs of splenomegaly – in 14.8%. Secondary to administration of medicinal product, HBeAg were detected in 11% of patients and HBV DNA was undetectable in 30% of children with CHB, which may indicate the decreased viremia. IFN-α level was increased 3-fold compared with the baseline in children with CHB and in patients with CHC – 4.2-fold.

11	Kriuchko T. A., Mohammed M. A. Abdalaal, 2006 [23]	54/24	Results of the serological test in children after 6-month treatment with the medicinal product PROTEFLAZID® demonstrate the significantly decreased anti-HCV IgM, decreased HCV RNA and increased IFN- α level. Parameters of the peripheral hemodynamics were normalized: decreased resistance index, pulsation index, increased volumetric blood flow and maximum flow velocity in the portal vein.
12	Nesyna I. M., 2006 [21]	111/27	PROTEFLAZID® is associated with the decreased severity of the clinical signs of CHB with the concomitant normalization of the biochemical and virological parameters (stable remission in 44.4% of patients), positive changes of interferon status parameters –IFN- α level increased by 3.5 times and IFN- γ level – by 1.4 times versus pretreatment values.
13	Mohammed M. A. Abdalaal, 2006 [22]	108/24	Addition of medicinal product PROTEFLAZID® to the combination therapy in children with CHC leads to the primary remission in 57.1% of patients, restoration of the clinical and laboratory parameters, stabilization of the signs of fibroplastic changes and correction of the hepatic hemodynamic disorders.
14	Kriuchko T. A., Nesyna I. M., 2006 [20]	47/27	Secondary to administration of medicinal product PROTEFLAZID®, regression of the cytolytic syndrome symptoms was observed: decreased total bilirubin and its fractions compared to the pre-treatment values (36.5 ± 3.7 $\mu\text{mol/l}$ versus 24.0 ± 1.61 $\mu\text{mol/l}$; 14.35 ± 1.87 $\mu\text{mol/l}$ versus 8.1 ± 0.88 $\mu\text{mol/l}$; 22.15 ± 2.11 $\mu\text{mol/l}$ versus 15.9 ± 0.89 $\mu\text{mol/l}$); normalized transaminase activity (ALT 0.83 ± 0.09 $\mu\text{mol/h}$ versus 0.6 ± 0.04 mmol/h ; AST 0.5 ± 0.06 mmol/h versus 0.37 ± 0.03 mmol/h). Therapy facilitates the restoration of the hepatic functions enhancing the bile secretion, normalizes lipid, carbohydrate and protein metabolisms.
15	Kriuchko T. A., Nesyna I. M., 2007 [24]	50/20	Flavozid® was associated with the clinical improvement in 80% of patients: normalized pigment metabolism and decreased enzyme activity. Relief of the pain syndrome occurred earlier than in the reference group (basic therapy). The immunity status tests performed secondary to administration of the medicinal product demonstrated the significant changes in the lymphocyte subpopulation (increased count of the initially decreased T-helpers, immunoregulatory index and natural killers). IFN- γ concentration was increased.
16	Kriuchko T. A., 2008 [25]	71/41	Medicinal product Flavozid® facilitates the restoration of the hepatic functions. It enhances the bile secretion, normalizes the lipid, carbohydrate and protein metabolisms. IFN- α concentration in children who received the medicinal product was 3.5-fold higher than the pre-treatment value and almost 2-fold higher than the value in the reference group (basic therapy). IFN- γ concentration was increased 1.4-fold versus 1.1-fold in the reference group compared with the pre-treatment concentration.

Treatment of hepatitis B in pregnant			
17	Mitsoda R. M., 2005 [27]	164/26	PROTEFLAZID® added to the basic therapy in pregnant with the active replication of HBV results in the decreased rate of preterm and rapid labor and decreased blood loss during delivery.
18	Mitsoda R. M., 2006 [28]	99/35	Medicinal product PROTEFLAZID® used in women with the replication causes the decrease of hyperthermia rate (31.4 versus 35.9 in the reference group [basic therapy]) and total number of anemias of different severity (28.6 versus 36.0 in the reference group). The postpartum period in 51.4% of the parturient woman was without complications compared with 32.8% in the reference group.
19	Mitsoda R. M., 2006 [30]	99/49	PROTEFLAZID® used in the combination therapy during the pregnancy facilitates the 2-fold decrease of the asphyxia rate and 2.5-fold decrease of the acute ischemic injury of CNS compared with the reference group (basic therapy).
20	Mitsoda R. M., 2007 [29]	1158/42	PROTEFLAZID® (compared with the reference group [basic therapy]) allows decreasing the threatened miscarriage rate (by 28.4%); preterm delivery rate by 5 times and rapid labor rate by 1.4 times; decreasing the period without amniotic fluid and blood loss ensuring the reduction of the inpatient days (by 0.4 days); newborns of the women who received the medicinal product are half as likely to be born asphyxiated and 2.5 times less often with the acute ischemic injury of the central nervous system.
21	Pekhno N. V., 2005 [33]	150/50	Administration of medicinal product PROTEFLAZID® for the placental insufficiency correction had the positive effect on the microcirculation in the pregnant women with a history of HBV. When compared with the control group (standard therapy: vitamins, hepatoprotectors) the following was observed: improved condition of the microcirculatory vessels during the pregnancy, decreased rate of threatened miscarriage from 22% to 10%, anemia – from 38% to 18%; premature rupture of the fetal membranes – from 46% to 20%; intrauterine growth retardation – from 40% to 22%; the medicinal product allows decreasing the perinatal pathology level in pregnant women with a history of HBV by half (from 40% to 20%).

22	Zarzhova Z. Sh., 2008 [31]	80/30	PROTEFLAZID® had the positive effect on the intrauterine fetal condition; cardiotocography and Doppler ultrasound findings were improved by 2.5 times compared to the reference group (standard therapy). It allows decreasing the birth rate of children with the pathology by 2 times, improving the adaptation period by 2 times and lowering the risk of transmitting infection to children.
23	Zaripova Z. Sh., 2008 [32]	30/30	The morphometry findings showed the improved blood supply to the placental tissue and resolved stromal edematous events secondary to CHB treatment with the medicinal product PROTEFLAZID®. Vasodilation leads to the increased oxygen supply to the cellular elements, which was accompanied by 2-fold decrease of the syncytiotrophoblast volume with the mean value of $9.6 \pm 1.7\%$. Area of fibrinoid ($4.8 \pm 0.9\%$) and calcinosis ($1.7 \pm 0.3\%$) was reduced secondary to the treatment.

In total, 2 756 patients took part in the 23 analyzed clinical studies, 1 021 of them received medicinal products PROTEFLAZID® (drops) or Flavozid® (syrup). Authors of all above mentioned studies emphasize the positive clinical and laboratory changes over time in patients who received the mentioned medications.

Special attention is drawn to the use of medicinal product PROTEFLAZID® in pregnant women with a history of hepatitis B or with hepatitis during the pregnancy. PROTEFLAZID® added to the basic therapy of hepatitis allows decreasing the threatened miscarriage rate (by 28.4%); preterm delivery rate by 5 times and rapid labor rate by 1.4 times; decreasing the period without amniotic fluid and blood loss ensuring the reduction of the inpatient days; newborns of the women who received the medicinal product are half as likely to be born asphyxiated and 2.5 times less often with the acute ischemic injury of the central nervous system. Administration of the medicinal product is associated with the improved condition of the microcirculatory vessels during the pregnancy, decreased rate of threatened miscarriage from 22% to 10%, anemia – from 38% to 18%; premature rupture of the fetal membranes – from 46% to 20%; intrauterine growth retardation – from 40% to 22%. The medicinal product allows decreasing the perinatal pathology level in pregnant women infected with HBV by half (from 40% to 20%) and lowering the risk of HBV transmission to the children.

Conclusions

The preclinical studies have demonstrated that PROTEFLAZID® effectively inhibits HCV RNA expression and surrogate BVDV reproduction both in single-agent infection and in the mixed infection model (HIV + HCV). It was proved that the medicinal product is the inducer of α - and γ -IFN both in vitro and in vivo. In addition to the antiviral activity, PROTEFLAZID® exerts the antioxidant and apoptosis-modulating properties.

The systemic analysis of 23 independent clinical studies involving more than 1000 patients has demonstrated the matching positive effect of outcome of treatment of the acute and chronic viral hepatitises using the medicinal product PROTEFLAZID® (drops) and medicinal product Flavozid® (syrup) derived from it and confirmed that the above mentioned products exert the antiviral, immuno-modulating, antioxidant and apoptosis-modulating properties detected at the preclinical studies.

Thus, the medicinal products PROTEFLAZID® (drops) and Flavozid® (syrup) are effective and safe agents used in the clinical settings for treatment of the acute and chronic hepatitis B and C in different groups of patients (adults, children of different age).

The results of the successful clinical studies supported the addition of medicinal products PROTEFLAZID® (drops) and Flavozid® (syrup) to the standard regimens of treatment of the viral hepatitis in the patients from different categories; they are presented in some guidelines and information letters from the Ministry of Health of Ukraine [34–43].

References:

1. EASL (2014) EASL Clinical Practice Guidelines: Management of hepatitis C virus infection. Journal of Hepatology, vol. 60, no 2, pp. 392–420.
2. Rybalko S. (ed.) (2003) Otchet o nauchno-issledovatel'skoj rabote provedenie dopolnitel'nyh doklinicheskikh ispytanj novyh form preparata Proteflazid® (Neoflazid) [Report on the scientific research: conduction of additional preclinical testing of new forms of drug Proteflazid® (Neoflazid)]. Kyiv.
3. Starosila D. (2014) Vlastivosti novih spoluk z roslinnih flavonoidiv ta mehanizmi ih antivirusnoi dii [Properties of novel substances based on plant flavonoids and mechanisms of their antiviral activity]. (PhD Thesis), Kyiv.
4. Rybalko S. (ed.) (2004) Otchet o provedenii dopolnitel'nyh doklinicheskikh ispytanj preparata Proteflazid na modeli virusa gepatita C [Report on additional pre-clinical trials of the drug Proteflazid on the model of hepatitis C virus]. Kyiv.
5. Porva Y. (2010) Modelyuvannya infekcii virusnogo gepatitu C *in vitro* ta vivchennya antivirusnoi aktivnosti preparativ grupi flavonoidiv i elagotanimiv [Viral hepatitis C infection *in vitro* modeling and study of antiviral activity of group preparations of flavonoid and elagotanine]. (PhD Thesis), Kyiv.
6. Palchikovska L., Vasilchenko O., Platonov M. (2013) Antivirusni vlastivosti roslinnih flavonoidiv – inhibitoriv sintezu DNK i RNK [Antiviral properties of herbal flavonoids – inhibitors of DNA and RNA synthesis]. Biopolymers and Cell, vol. 29, no 2, pp. 150–156.
7. Rybalko S. (ed.) (2010) Otchet ob izuchenii mehanizmov dejstviya biologicheskii aktivnyh veshhestv lechebnoj substancii «Proteflazid» [The report on the study of the mechanisms of action of bioeffecting agents of therapeutic substance «Proteflazid»]. Kyiv.
8. Zavelevich M., Dyadyun S., Ribalko S. (2002) Interferonogenna ta apoptozmodulyuyucha aktivnist' preparatu proteflazid [Interferonogenic and apoptosis-modulating activity of the drug proteflazid]. Visnik Vinnickogo derzhavnogo Universitetu, no 6 (2), pp. 281–283.
9. Matyash V. (ed.) (2000) Zvit po klinichnomu doslidzhennyu «Vidkrite doslidzhennya po vivchennyu efektyvnosti ta perenosimosti preparatu Proteflazid, virobnictva TOV «Ekofarm» u hvori virusnim gepatitom B ta C (2-ga faza) [The report on clinical study «Open research on the study of the efficacy and tolerability of Proteflazid, produced by «Ecopharm» Ltd in patients with hepatitis B and C (2 nd phase)]. Kyiv.
10. Matyash V., Shevchuk V., Tokunova T., Atamanyuk V. (2002) Terapevtichna efektyvnist' Proteflazidu pri virusnih (C ta B) gepatitah [Therapeutic efficacy of Proteflazid in viral (C and B) hepatitis]. Visnik Vinnickogo Derzhavnogo universitetu, no 2, pp. 313–314.
11. Matyash V., Bobrova I., Shevchuk V. et al. (2002) Farmakoterapevticheskie mehanizmy Proteflazida pri virusnyh gepatitah [Pharmacotherapeutic mechanisms of Proteflazid in viral hepatitis]. Problemy epidemiologii, diagnostiki, kliniki, lechenie i profi laktika infekcionnyh boleznej [Problems of

- epidemiology, diagnosis, clinical features, treatment and prevention of infectious diseases]. Kyiv, pp. 381–384.
12. Matyash V. (ed.) (2003) Zvit pro naukovo-doslidnu robotu «Rozrobka novih metodiv etiopatogenetichnoï terapiï virusnogo gepatitu B ta C» [The report on research work «Development of new methods for ethiopathogenetic therapy of hepatitis B and C»]. Kyiv.
 13. Matyash V., Bobrova I., Shevchuk V., Vlasik T. (2004) Ocinka terapevtichnoï efektyvnosti Proteflazidu pri gostromu ta hronichnomu virusnomu gepatiti B [Assessment of therapeutic efficiency of Proteflazid in acute and chronic viral hepatitis B]. *Nova medicina*, vol. 17, no 6, pp. 36–39.
 14. Ivahiv O., Andrejchin M., Vishnevs'ka N. (2004) Klinichna efektyvnist' Proteflazidu i cikloferonu pri hronichnih gepatitah B ta C [Clinical efficiency of Proteflazid and Cycloferon in chronic hepatitis B and C]. *Proceedings of the Virusni hvorobi. Toksikoz. Hlamidioz (Ternopil, Ukraine, May 5–6, 2004)*. Ternopil, p. 26.
 15. Nartov P., Volobueva O. (2003) Proteflazid v kompleksnom lechenii bol'nyh s ostrym virusnym gepatitom B [Proteflazid in the complex treatment of patients with acute viral hepatitis B]. Paper presented at *Virusnye gepatity v praktike terapevta, semejnogo vracha i infekcionista. Sovremennye metody diagnostiki i terapii*. Kharkiv, Ukraine, March 11–12, 2003.
 16. Dikij B., Pyurik V., Ostyak R. (2003) Primenenie Proteflazida v kompleksnom lechenii bol'nyh gepatitom B [The use of «Proteflazid» in complex treatment of patients with hepatitis B]. *Proceedings of the Upravlyayemye infekcii (Ivano-Frankivsk, Ukraine, May 14–15, 2003)*. Ivano-Frankivsk, pp. 73–74.
 17. Savellev V., Bondareva V. (2005) Efektyvnist' zastosuvannya proteflazidu u hvorih na virusni gepatiti [Efficiency of proteflazidum application in patients with viral hepatitis]. *Proceedings of the Himioterapiya ta imunokorekciya infektsijnih hvorob (Ternopil, Ukraine, May 7–10, 2005)*. Ternopil, pp. 85–86.
 18. Kryuchko T., Nesina I. (2002) Effektivnost' primeneniya Proteflazida v kompleksnoj terapii detej s hronicheskim gepatitom B [Efficiency application of Proteflazid in the complex therapy of children with chronic hepatitis B]. *Imunologiya ta alergologiya*, no 4, pp. 38–40.
 19. Kryuchko T., Nesina I., Mohammed M.A. Abdalaal. (2005) K voprosu farmakoterapii detej s hronicheskimi virusnymi gepatitami B i C [On the question of pharmacotherapy in children with chronic viral hepatitis B and C]. *Proceedings of the Vnutrikletochnye infekcii i sostoyanie zdorov'ya detej v XXI veke (Donetsk, Ukraine, October 25–26, 2005)*. Donetsk, p. 7.
 20. Kryuchko T., Nesina I. (2006) Zastosuvannya induktora endogenного interferonu v protokolah likuvannya ditej z hronichnim gepatitom B [Application of an inducer of endogenous interferon in treatment protocols of children with chronic hepatitis B]. *Sovremennaya pediatriya*, vol. 11, no 2, pp. 102–105.
 21. Nesina I. (2006) Diagnostichno-prognostichne znachennya kliniko-laboratornih pokaznikov u ditej z hronichnim gepatitom B [Diagnostic-prognostic value of the clinico-laboratory rates in children with chronic hepatitis B]. (PhD Thesis), Kyiv.
 22. Mohammed M.A. Abdalaal. (2006) Kliniko-gemodinamichni porushennya ta ih korekciya u ditej z hronichnim virusnim gepatitom C [Clinicohemodynamic disorders and their correction in children with chronic viral hepatitis C]. (PhD Thesis), Kyiv.
 23. Kryuchko T., Mohammed M.A. Abdalaal. (2006) Primenenie Proteflazida v kompleksnoj terapii detej s hronicheskim gepatitom C [Application of «Proteflazid» in complex therapy of children with chronic hepatitis C]. *Reproduktivnoe zdorov'e zhenshhiny*, vol. 26, no 2, pp. 200–203.

24. Kryuchko T., Nesina I. (2007) Terapevticheskaya effektivnost' protivovirusnogo preparata «Flavozid» v lechenii hronicheskikh gepatitov virusnoj etiologii u detej [The therapeutic efficacy of the antiviral drug «Flavozid» in the treatment of chronic hepatitis of viral etiology in children]. *Sovremennaya pediatriya*, vol. 14, no 1, pp. 62–65.
25. Kryuchko T. (ed.) (2008) Zvit pro provedennyya klinichnogo doslidzhennyya «Ocinka effektivnosti ta perenosimosti preparatu Flavozid u ditej z hronichnimi gepatitami B ta C [Report on conducted clinical study «Evaluation of the efficacy and tolerability of «Flavozid» in children with chronic hepatitis B and C»]. Poltava.
26. Kaminskyi V., Shalko M., Grynevych O. (2014) Proteflazid u vagitnih: sistemnij oglyad rezul'tativ pislyareestracijnih sposterezhen' za bezpechnistyu ta effektivnistyu zastosuvannya [Proteflazid in pregnant women: systematic review of observing of post-registration safety and efficacy]. *Zdorov'e zhenshhiny*, vol. 92, no 6, pp. 160–164.
27. Mitsoda R. (2005) Rody u zhenshhin s aktivnoj replikaciej vzbuditelej virusnyh gepatitov vo vremya gestacii i korekcii oslozhnenij Proteflazidom [Childbirth in women with active replication of pathogens of viral hepatitis while being in gestation and correction of complications Proteflazid]. *Reproduktivnoe zdorov'e zhenshhiny*, no 4, pp. 77–80.
28. Mitsoda R. (2006) Poslerodovoj period u zhenshhin s replikaciej vzbuditelej virusnyh gepatitov vo vremya gestacii i korekcii oslozhnenij Proteflazidom [Postpartum period in women with replication of pathogens of viral hepatitis while being in gestation and correction of complications Proteflazid]. *Reproduktivnoe zdorov'e zhenshhiny*, vol. 25, no 1, pp. 154–156.
29. Mitsoda R. (2007) Patogeneticheskie osobennosti, prognozirovanie, profi laktika i lechenie akusher-skich oslozhnenij u zhenshhin, kotorye stradali ili stradayut virusnymi gepatitami [Pathogenetic features, prognostication, prophylaxis and medical treatment of obstetric complications at women who suffered or suffer from viral hepatitis] (MD Thesis). Kyiv.
30. Mitsoda R. (2006) Rannij neonatal'nyj period u novorozhdennyh ot zhenshhin s replikaciej vzbuditelej virusnyh gepatitov vo vremya gestacii i korekcii oslozhnenij [Early neonatal period in newborns of women with replication agents of viral hepatitis while being in gestation and correction of complications]. *Reproduktivnoe zdorov'e zhenshhiny*, vol. 26, no 2, pp. 138–141.
31. Zarzhova Z. (2008) Sostoyanie ploda i novorozhdennyh, rozhdennyh ot materej, stradayushhih hronicheskimi virusnymi gepatitom B minimal'noj stepeni aktivnosti [The fetal status and newborn, was born to mothers with chronic viral hepatitis in the minimal degree of activity]. *Novosti dermatologii i reproduktivnogo zdorov'ya*, no 3, pp. 29–31.
32. Zaripova Z., Israilov R., Kurbanov S. (2008) Patomorfometricheskaya kartina placenty zhenshhin s hronicheskimi virusnymi gepatitom B, lechennyh preparatom Proteflazid [Patomorfometric status of the placenta of women with chronic hepatitis B treated by drugs Proteflazid]. *Novosti dermatologii i reproduktivnogo zdorov'ya*, no 1, pp. 29–31.
33. Pehnio N. (2005) Korekciya placentarnoy nedostatnosti u vagitnih z virusnim gepatitom B u anamnezi [Correction placental insufficiency in pregnant women with virus hepatitis B in anamnesis] (PhD Thesis), Kyiv.
34. Odessa State Medical University (2002) Diagnostika, lechenie, profi laktika i dispanserizaciya vzroslyh i detej, bol'nyh virusnymi gepatitami [Diagnosis, treatment, prevention and clinical examination of adults and children with viral hepatitis]. Odessa.
35. Tashkent Institute Postgraduate Medical (2004) Proteflazid v lechenii ostryyh i hronicheskikh virusnyh gepatitov [Proteflazid in the treatment of acute and chronic viral hepatitis]. Tashkent.

36. National Academy of Medical Sciences of Ukraine. (2005) Etiopatogenetichna terapiya gostrih ta hronichnih gepatitiv B ta C [Etiopathogenetic treatment of acute and chronic hepatitis B and C]. Kyiv.
37. Ministry of Healthcare of Ukraine (2006) Akushers'ki uskladnennya pri virusnih gepatitah (Integral'na ocinka, profilaktika ta likuvannya). Rekomendacii MOZU [Obstetric complications of viral hepatitis (Integral assessment, prevention and treatment). Guidelines of MHCU]. Kyiv.
38. Ministry of Healthcare of Ukraine (2002) Metodika kompleksnoi terapii Proteflazidom ta roztoropshoyu plyamistoyu gostrogo virusnogo gepatitu B ta C. Informacijnij list MOZU [Methods of complex therapy Proteflazid and Silybum mariánum acute viral hepatitis B and C. Fact sheet of MHCU]. Kyiv.
39. Ministry of Healthcare of Ukraine. (2002) Metodika primeneniya kompleksnoj terapii Proteflazidom i plazmaferezom pri virusnom gepatite «C». Informacionnoe pis'mo MOZU [The method of application of complex therapy Proteflazid and plasmapharese in viral hepatitis «C». Fact sheet of MHCU]. Kyiv.
40. Ministry of Healthcare of Ukraine. (2002) Metodika zastosuvannya preparatu Proteflazid v terapii virusnogo gepatitu C. Informacijnij list MOZU [The method of application of Proteflazid in the treatment of viral hepatitis «C». Fact sheet of MHCU]. Kyiv.
41. Ministry of Healthcare of Ukraine. (2004) Metodika zastosuvannya preparatu «Proteflazidu» pri gostromu virusnomu gepatiti B. Informacijnij list MOZU [The method of application of Proteflazid in acute viral hepatitis B. Fact sheet of MHCU]. Kyiv.
42. Ministry of Healthcare of Ukraine. (2006) Zastosuvannya preparatu «Proteflazid» v kompleksnij terapii ditej z hronichnim gepatitom C. Informacijnij list MOZU [Application of the drug «Proteflazid» in the treatment of children with chronic hepatitis C. Fact sheet of MHCU]. Kyiv.
43. Ministry of Healthcare of Ukraine. (2006) Zastosuvannya preparatu «Proteflazid» v kompleksnij terapii ditej z hronichnim gepatitom B. Informacijnij list MOZU [Application of the drug «proteflazidum» in the treatment of children with chronic hepatitis B. Fact sheet of MHCU]. Kyiv.

Reference: A. M. Pechinka, O. Y. Hrynevych, T. A. Kryuchko, V. R. Shaginan, L. M. Solomakha. *Proteflazif: the specific activity against the hepatitis C virus in preclinical studies; efficacy and safety in the treatment of hepatitis B and C in clinical practice (systematic review) // Clinical Infectology and Parasitology. – 2015. – No. 2 (13). – pp. 78–97.*



Section 6

6.1 PROTEFLAZID® IN PREGNANT WOMEN: SYSTEMATIC REVIEW OF OBSERVING OF POSTREGISTRATION SAFETY AND EFFICACY

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Abstract

The article presents the results of literature analysis, the experience of using «PROTEFLAZID®» to treat various diseases during pregnancy. This scientific review is based on post-marketing studies and comparative clinical observations. The safety and efficacy of using «PROTEFLAZID®» during treatment of viral and viral-bacterial infections, reducing the incidence of obstetric and perinatal complications in pregnant women are proved in the study.

Key words: PROTEFLAZID®, pregnancy, immune deficiency, infection, fetus.

Introduction

Prevention and treatment of the viral and viral and bacterial infections play a special role in the medical practitioners due to their prevalence. It is well known that currently 80–90% of population is infected with herpes simplex virus and up to 20% – with cytomegalovirus. The trend towards the increased incidence of viral hepatitis is continued to be observed. Epidemics of the acute respiratory viral infections occur almost every year. These diseases are especially dangerous during the pregnancy, which is known to be associated with the natural immunosuppression and, therefore, the decreased body defenses and its ability to fight these diseases. In women who have suffered from these diseases during pregnancy, the significantly increased incidence of the following complications is observed – threatened miscarriage, fetoplacental insufficiency, polyhydramnios, oligohydramnios. Children born in such mothers have lower physical development indices and are more likely to fall ill during the first years of life.

Treatment of women during the pregnancy is associated with certain difficulties as it is impossible to administer many classes of medicinal products that are prohibited or not recommended for use in pregnant women. At the same time, the medicinal products that exert both antiviral and immunomodulatory activity and are allowed to be used during gestation can not only increase the treatment efficacy of certain diseases caused by viruses but also, due to it, decrease the incidence rate of the obstetric and perinatal complications of pregnancy in such women.

One of such medicinal products is PROTEFLAZID® (drops) – the original national (SMC “Ecopharm” LTD, Ukraine) herbal product containing the flavonoid glycosides obtained by the alcoholic extraction from *Deschampsia caespitola* L. and *Calamagrostis epigeios* L. grasses with the oxygen-containing heterocycle composing the basis of their molecules. Preclinical and clinical studies of active substances of PROTEFLAZID® extract conducted in the institutes of National Academy of Sciences, National Academy of Medical Sciences and Ministry of Health of Ukraine (R. E. Kavetskyi Institute of Experimental Pathology, Oncology and Radiobiology, D. K. Zabolotnyi Institute of Microbiology and Virology, L. V. Hromashevskyi Institute of Epidemiology and Infectious Diseases, P. L. Shupyk National Medical Academy of Postgraduate Education, Odesa, Donetsk, Zaporizhzhia, Lviv, Uzhhorod Medical Universities) detected that the medicinal product possesses the following pharmacodynamic properties:

1. Antiviral effect involves the inhibition of the virus-specific enzymes – thymidine kinase, DNA polymerase and reverse transcriptase in the virus-infected cells that is associated with the reduced or complete suppression of viral protein replication and, as a consequence, prevent viruses from replicating.
2. Immunomodulating effect is associated with the increased production of the endogenous α - and γ -interferons up to the physiological level of the individual body; stimulation of the nonspecific body resistance involving the increased scavenging, engulfing and digesting functions of macrophages.
3. Antioxidant effect is associated with the increased resistance of cells and tissues to the damaging effects of free-radical oxidation products, as its level increases in most pathological processes, especially in inflammation.
4. Apoptosis-modulating properties are associated with the more rapid transition of virus-infected cells into apoptosis stage facilitating faster elimination of infected cells from the body.

As medicinal product PROTEFLAZID® possesses both antiviral and immunomodulating properties, it justifies its broad use in the various areas of medicine for the prevention and treatment of a number of infectious diseases (viral hepatitis, type 1 and 2 herpes virus, cytomegalovirus, human papillomavirus infection).

Immunomodulating effect of medicinal product PROTEFLAZID® allows using it in the combination therapy of the bacterial and fungal infections. In gynecological practice, the medicinal product is also used for irrigations and applications at the affected areas of mucous membranes in the cervical herpetic and papillomavirus lesions and in the treatment of colpitis and vaginitis.

Preclinical experimental studies of the acute, subacute and chronic toxicity of PROTEFLAZID® demonstrated that the medicinal product belongs to toxicity class IV of the generally accepted classification (compounds with a low level of toxicity). Safety studies of its use in the pregnant animals did not detect embryotoxic, embryolethal or teratogenic effects. The medicinal product also has no effect on the reproductive function of the experimental animals of different species.

However, despite the long-term experience in using the medicinal product PROTEFLAZID® in medical practice, the possibility of its use during pregnancy raises a number of questions for many doctors including obstetricians and gynecologists and, of course, patients, first of all regarding its possible use at the different gestational stages, possible effect on the fetal condition and postnatal development of the child.

It is explained not only by the particular caution of doctors and patients against the use of medications during pregnancy in general but also, especially, by the contraindications of many antiviral and immunotropic products to their use in this period (Rimantadine, Ganciclovirip, immune serums, etc.) or by the fact that currently such experience is insufficient to ensure the complete maternal and fetal safety.

This scientific review is devoted to the analysis of the available literature sources pertinent to the use of medicinal product PROTEFLAZID® for the treatment of different diseases during pregnancy; the review was performed based on the post post-marketing comparative and observational studies.

Major part of the literature publications is devoted to the clinical use of medicinal product PROTEFLAZID® in the opportunistic infections that are developed in the immune deficiency. The herpesvirus, cytomegalovirus and chlamydia are the leading infections among them; according to the national and foreign literature sources, more than 90% of female population is infected with them both in our country and abroad.

Viral and bacterial infections are known to be among the main reasons of the complicated pregnancy. In particular, according to Nahornaia V. F., Nikolaieva S. V. [19], they are the prevailing cause of more than 58% of miscarriage cases; herpesvirus (HV) infections are the most common among them (16.67%); in 41% of cases they are associated with other sexually transmitted infections (cytomegalovirus [CMV], chlamydia, ureaplasma, toxoplasma, trichomoniasis); however, in the bacterial and viral associations, the priority is given to HV – 60.9% and CMV – 37.5%. The reasons involve not only the significant proportion of patients infected pre-pregnancy (i.e. pregnancy occurs secondary to the pathological changes in the woman's body caused by the infectious agent) but also well-known immunosuppression caused by pregnancy due to which a significant number of infected women in remission is transferred to the active phase of disease giving rise to the pregnancy complications (threatened miscarriage, spontaneous miscarriage, polyhydramnios, fetal growth retardation, impaired postnatal adaptation period of newborns, etc.).

Analysis of management of 267 such patients performed by Nagornaia V. F., Nikolaieva S. V., Posokhova S. P., Humeniuk L. I. [26] demonstrated that administration of medicinal product PROTEFLAZID® both in the exacerbated HV-infection during pregnancy and in the periconception period intended to prepare the infected women for the pregnancy is pathogenetically justified not only due to the product property to exert the antiviral effect, but also to improve the immune responsiveness increasing the duration of HV infection remission and reducing the negative impact of viremia on the fetal condition and the fetal-placental complex.

Similar data were obtained by Simrok V. V. and Hordienko O. V. [25] who used PROTEFLAZID® systemically and topically in the combination therapy of HV and CMV infections in females with a history of perinatal losses associated with the chronic genital inflammation. The high efficacy of PROTEFLAZID® used in the pregravid preparation and treatment of such patients is also demonstrated by the fact that when pregnancy occurred in such women, hydramnios was observed 2.5 times less often and threatened miscarriage – 2 times less often both in the early and late stages.

The authors did not observe exacerbation of the pregnancy-related infection signs in any patient while disease manifestation was recorded almost in 20% of patients who received the

basic therapy (without medicinal product PROTEFLAZID®). Further, the authors emphasize the good tolerability of medicinal product PROTEFLAZID® and report no side effects.

Vdovychenko Yu. P., Baskakov P. N., Hlazkov I. S. [7] also recommend using PROTEFLAZID® in the latent and recurrent herpetic infection. The authors believe that one 21-day therapy course is sufficient in the latent infection but in the recurrent infection 2–3 courses are needed. The use of this method allowed reducing incidence of threatened miscarriage from 73.3% to 36.7%, fetoplacental insufficiency – from 46.7% to 23.3%, preeclampsia of different severity – from 23.3% to 10.0% and preterm delivery – from 16.7% to 6.7%.

Property of medicinal product PROTEFLAZID® to increase the nonspecific immune resistance (in particular to stimulate the production of alpha- and gamma-interferons) supported its use as immunomodulating therapy in the pregravid preparation of women with the urogenital infection and with a history of miscarriage within the past 6–12 months. Medicinal product PROTEFLAZID® was systemically used in addition to the medications that help modifying the genital biocenosis in 50 such women who had threatened miscarriage in gestation weeks 8–10 and gestation weeks 18–22 [16]. The authors performed the analysis of main changes of rate of certain microorganism species isolation in the cervical canal and urethra of women who received the medicinal product PROTEFLAZID® in addition to the standard therapy and determined that its effect resulted in the significantly decreased levels of contamination with chlamydia (62.0% versus 86% in the reference group), trichomonads (36% and 50%, respectively), mycoplasma (34% and 52%, respectively), ureaplasma (28% and 44%), fungi (Candida) and viruses. The significant improvement of the genital biocenosis condition allowed, in its turn, reducing the rate of spontaneous abortion by 3.3 times, preterm delivery – by 2.9 times, neonatal asphyxia – by 2 times, intrauterine infection – by 2.8 times and neonatal hypoxic encephalopathy – by 2.1 times.

Similar data was obtained when medicinal product PROTEFLAZID® was used in 55 females with the urogenital inflammatory diseases at the stage of their preparation to the pregnancy and in 55 females with the threatened miscarriage secondary to the same infection [5]. The results of the bacteriological tests of genital discharge of such women proved that addition of medicinal product PROTEFLAZID® to the combination therapy ensures the significantly decreased incidence of the genital and urinary infections. As a result, it allows reducing the incidence of pregnancy and birth complications in such women, improving functions of fetoplacental complex, fetal and neonatal condition and development of children in the early neonatal period.

In Ivano-Frankivsk National Medical Academy, Henyk N. I. [12] conducted the clinical study of efficacy of medicinal product PROTEFLAZID® used in the combination therapy of pregnancy complications in women with chlamydia infection, as it is one of the main causes (according to some authors, it accounts up to 27.4%) of antenatal fetal death. The generalized infections (pneumonia, meningoencephalitis) are observed in 60–70% of neonates born in such mothers, conjunctivitis – in 35–50%, respiratory and lung syndromes – in 12–20%. PROTEFLAZID® was added to the basic therapy with erythromycin (daily dose: 2 g, therapy duration: 10 days) in 30 pregnant women with the laboratory confirmed chlamydia infection.

In the opinion of the author, the possibility of using this medicinal product is explained not only by its antiviral effect, but also, above all, by its property to increase the production of endogenous alpha- and gamma-interferons and thus restore the activity of macrophage cells with subsequent modulation of the cellular immunity condition and the

induction of reparative and regenerative processes. The author performed the detailed analysis of the main parameters of cellular and immune immunity in the women who received the medicinal product PROTEFLAZID® during pregnancy and made a conclusion that the product ensures the marked immunomodulating effect manifested as the increased total count of T-lymphocytes and B-lymphocytes. At the same time, the population composition of T-cells was normalized; it was manifested by almost 2-fold increase of the count of helper CD4+ cells secondary to the moderate decrease of CD8+ cells; after the treatment course with medicinal product PROTEFLAZID®, their blood counts were consistent with the counts in the apparently healthy pregnant women. Medicinal product PROTEFLAZID® also exerted the marked inhibitory effect on the complex formation decreasing it by 1.5 times.

Ostrovska O. N. [20] analyzed the condition of some components of the cellular immunity when medicinal product PROTEFLAZID® was used in the combination therapy of threatened miscarriage in 42 women with chlamydia infection and made a conclusion that the medicinal product ensures the restoration of cooperation mechanisms in the system of lymphocyte subpopulations and increases the production of gamma interferon. It improves the processes of eliminating the chlamydia pathogens from the affected cells and thereby contributes to the normalization of the immune status. Elimination of chlamydia from the body allows terminating the complete cascade of pathological mechanisms that ultimately cause miscarriage in the infected mothers. Special attention should be paid to some efficacy studies of PROTEFLAZID® used in the pregravid preparation of women with a history of premature termination of pregnancy or miscarriage. The chronic inflammatory genital process (salpingo-oophoritis) is known to be the cause of the miscarriage almost in 80% of women, endometritis and inflammatory cervical pathology – almost in 30%. Pylypenko O. N., Zhelieznaia A. A., Borysova Ya. Yu. et al. [21] developed the recommendations on how to increase the efficacy of such regimen of women preparation to the pregnancy using PROTEFLAZID® as the immunomodulating and antiviral product in the women with chlamydia infection. This pathogen is characterized by the intracellular location explaining the long asymptomatic course of this disease and extremely insufficient efficacy of antibiotics due to their low capability to be accumulated in the cells and ensure the bacteriostatic or bactericidal concentration capable of destroying the pathogen. The authors used the method developed by them and involving medicinal product PROTEFLAZID® in 128 women with a history of miscarriage. They demonstrated that when this method was used, the etiological recovery occurs in 97.2% of cases, in contrast to 42.5% of women who received antibiotic monotherapy alone. No recurrences of chronic adnexitis were recorded for a long time in 87.4% of women who received the combination of antibiotic and PROTEFLAZID®. All women who received treatment by this method had pregnancies without perinatal losses.

Efficacy of medicinal product PROTEFLAZID® used as a component of antiviral therapy at the preconception preparation in women infected with the herpes simplex virus and CMV was also proven.

According to Simrok V. V., Hordienko O. V. [25], using this medicinal product in the comprehensive treatment of HV and CMV infections in women just before the pregnancy facilitates the stabilization of immunoglobulin G at the normal levels in most subjects (8 of 11 patients) secondary to the absence of immunoglobulin M in all 11 women indicating absence of the disease recurrence due to the sufficient immune support of the body. It should

be noted that in future, pregnancy in such women proceeds with a lower complication rate – manifestations of chronic placental insufficiency and chronic fetal hypoxia were observed quite less frequently. No congenital manifestations of HV or CMV infection were observed in the newborns in any observation and titer levels of M and G antibodies to those infections in all newborns were consistent with the maternal levels.

Specialists from the Research and Practical Center of Obstetrics and Gynecology of the Ministry of Health of Uzbekistan also informed about their positive experience of using medicinal product PROTEFLAZID® in pregnant women with a history of antenatal fetal death [1]; they used that medicinal product in I–III trimesters in 30 women with the threatened miscarriage and serologically verified infection with type I and type II human herpes simplex viruses and proved that using the medicinal product allowed decreasing the incidence of cold-related and viral diseases by 1.5 times, incidence of preeclampsia – by 1.3 times, incidence of oligohydramnios, polyhydramnios and fetal growth retardation syndrome – 2–2.5 times.

The positive results obtained by the investigators when using medicinal product PROTEFLAZID® in pregnant women with HV, CMV and Chlamydia infections allowed introducing this medicinal product to the guidelines of the Ministry of Health of Ukraine “Diagnosis and treatment of genital infections in pregnant women” (Kyiv, 2005) [14], as the product to be used to reduce the negative impact of such infection on the pregnancy course, delivery and the condition of the fetus and newborn.

Based on the experience in treatment of papillomavirus infection in pregnant women, Voloshyna N. N., in her clinical lecture [11] recommends such women to receive the comprehensive anti-inflammatory, antiviral and immunomodulating treatment starting from the second trimester considering the medicinal product PROTEFLAZID® to be perfect for this purpose, as it possesses all above mentioned properties. It is prescribed to be used as directed orally and topically (with tampons). As it is impossible to completely cure a woman from virus-carriage using modern available methods, **the authors considers the adequate pregravid preparation with medicinal product PROTEFLAZID® 3-4 months prior to conception to be the sufficient measure to prevent the exacerbation of papillomavirus infection during pregnancy.** As during delivery the transmission of human papillomavirus from mother to child can account for 97%, using this method of preventing infection exacerbations in pregnant women may significantly decrease the child infection rate and reduce the negative impact on their postnatal development. Such decrease was demonstrated in some clinical studies, in particular the study conducted by Hryshchenko O. V., Bobrytska V. V., Pak S. A. [13] who used PROTEFLAZID® in 33 pregnant women for 2–3 months with the repeated cycles and showed that already 5–7 days after treatment initiation, the regression of genital condylomas was started to be recorded; they disappeared completely in more than 60% of patients; in other women, the skin and mucosal lesions were reduced in size and no recurrences of the process or its progression were observed in any subject.

Clinically proven immunomodulating properties of medicinal product PROTEFLAZID® allowed its successful use to enhance the efficacy of conventional treatment of vulvovaginitis in pregnant women.

Insufficient efficacy of the standard therapy used in such women is explained, in particular, by the limited list of antimicrobial and antifungal products intended both for systemic and topical administration and allowed in pregnant women, especially in the first

trimester. According to Tanko O. P., Skorbach Yu. I. [27], addition of medicinal product PROTEFLAZID® to the combination therapy in 30 pregnant women with vulvovaginitis facilitated the tendency towards normalization of the enzymatic complement system, as evidenced by the increased complement titer compared with similar parameters pre-treatment, decreased content of the circulating immune complexes and increased level of IgG. At the same time, more rapid tendency towards normalization of the WBC and lymphocyte count and improvement of phagocytic activity was observed in the peripheral blood of such women secondary to the received treatment.

Another, very important clinical area of using the medicinal product PROTEFLAZID® in the pregnant women is treatment of influenza and acute respiratory viral infections. Other acute respiratory infections include parainfluenza, adenovirus infection, rhino- and respiratory syncytial virus infections and a number of other infections of viral etiology. In the patients who had influenza during pregnancy, higher incidence of miscarriage, congenital anomalies of fetal development and higher percentage of child mortality are observed. Such complications are most common when women are infected in the first and second trimesters, as they are not resistant to the infectious agents due to the pregnancy-associated immunosuppression. According to Duda O. K., Boiko V. O., Zhigarev Yu. O. [15], preterm delivery is observed in 16.5% of such patients. As the possibility of using a number of medicinal products from different pharmacological groups (antiviral, antimicrobial, antipyretic, non-steroidal anti-inflammatory, antihistamine, etc.), which are used to treat such patients in routine medical practice, is limited during pregnancy due to their possible negative impact on the pregnancy course, delivery, fetal condition and development and child postnatal development, the same authors consider the use of national herbal medicinal product PROTEFLAZID® to be justified in the pregnant women, as it possesses both antiviral, immunomodulating and antioxidant properties. Biological formula of this medicinal product ensures its direct antiviral effect both on DNA and RNA viruses at all stages of infection development. The medicinal product inhibits the virus-specific enzymes – DNA polymerase, thymidine kinase, reverse transcriptase and neuraminidase of influenza A and B viruses. As majority of viruses that cause the acute respiratory viral infections are RNA-containing (influenza, parainfluenza, coronaviruses, respiratory syncytial viruses, rhinoviruses) and some (adenoviruses) are DNA-containing viruses, property of medicinal product PROTEFLAZID® to inhibit some enzymes of such viruses allows its using in the broad range of inflammatory respiratory diseases caused by different pathogens [10].

Specifically, PROTEFLAZID® has been proven to exert the antiviral effect on the pandemic influenza A strain (H1N1/California/07/09) [28]. In addition, the immunomodulating effect of the product involves the inhibition of endogenous α - and γ -interferon synthesis with the immune system to be non-refractory. When a product is administered orally in the liquid dosage form (drops), it prevents the penetration of viruses into the body through the nasopharynx with the concomitant normalization of local immunity parameters of the upper respiratory tract, increasing the content of lysozyme and secretory immunoglobulin A in the nasopharyngeal mucus.

The use of the medicinal product PROTEFLAZID® is also supported by its antioxidant properties, as the fever and intoxication in the acute respiratory viral infections lead to the activation of the free-radical oxidation processes, which in their turn have the negative effect on the fetal condition and development.

Using the antiviral and immunomodulating properties of medicinal product PROTEFLAZID® also ensures decreased rate of perinatal complications in women with the viral hepatitis. Women with the active replication of hepatitis B and C pathogens occurring during gestation are at the particular risk due to the parenteral (and during pregnancy, trans-placental) route of infection characteristic to this disease, the significant incidence of the acute liver failure with encephalopathy and coma in the infected pregnant women and, as the consequence, the high mortality rate (3-fold higher in pregnant women). According to Mitsoda R. M. [17, 18] who analyzed the experience of using medicinal product PROTEFLAZID® in 99 women with the viral hepatitis B and C, including 35 patients with the active pathogen replication occurred during pregnancy, the incidence of pathological intrapartum blood loss was almost 3-fold decreased in the group of patients who received PROTEFLAZID®, with the lower rate of anemia of pregnancy, preterm delivery – by 2.2 times and rapid labor – by 3 times and the author especially emphasizes that inflammatory complications were much less common in the postpartum period; the author explains it by the immunomodulating properties of the medicinal product PROTEFLAZID®, as its use improves not only the systemic but also the local immunity, i.e. the body is able to resist the infectious agents. Obtained positive experience of using the medicinal product was the basis to include it to the regimens of prevention and treatment of the obstetric complications in viral hepatitis specified in the guidelines [2].

The articles of Vovk I. B. et al. [9]; Pylypenko O. N. [21]; Vakulenko H. A. et al. [6]; Serhienko S. M. [24]; Reznichenko N. A. et al. [22]; Reznichenko H. I. et al. [23]; Bilyk N. M. [3, 4] also support the high efficacy and safety of medicinal product PROTEFLAZID® used in the obstetric and gynecological practice.

Conclusions

Thus, analysis of the available literature sources pertinent to the results of clinical use of medicinal product PROTEFLAZID® both for prevention of the perinatal complications and treatment of women with the different viral and bacterial and viral diseases during pregnancy has demonstrated that the product was used with the significant clinical effect in more than 1000 patients in the different periods of pregnancy (trimesters I, II and III). All publications confirm that the antiviral, immunomodulating and antioxidant properties of medicinal product PROTEFLAZID® ensure the decreased rate of the perinatal complications in the gestational period associated with the concomitant diseases (viral hepatitis B and C, herpes, CMV, chlamydia infections, influenza and other acute respiratory viral infections). Efficacy of medicinal product PROTEFLAZID® used at the preconception preparation of the infected women to pregnancy has been proved. No adverse reactions were recorded during the long-term use of the medicinal product in the pregnant women. Considering the efficacy and safety of medicinal product PROTEFLAZID® in the pregnant women, its use was proposed and implemented in some guidelines of the Ministry of Health of Ukraine for treatment of the most common viral and bacterial and viral infections during pregnancy [2, 8, 14].

References:

1. Азімова Е. І. До питання профілактики рецидиву генітального герпесу у вагітних із антенатальною загибеллю плода в анамнезі / Е. І. Азімова, С. М. Султанов, Ф. П. Нішанова // *Здоров'я жінки*. – 2011. – Т. 58, №2. – С. 238–240.
2. Акушерські ускладнення при вірусних гепатитах (Інтегральна оцінка, профілактика та лікування) [Методичні рекомендації МОЗ України.] – К., 2006. – 33 с.
3. Білик Н. М. Діагностика та лікування ретрохоріальних та ретроплацентарних гематом у жінок з антифосфоліпідним синдромом та вірусною інфекцією / Н. М. Білик // *Педіатрія, акушерство та гінекологія*. – 2005. – №2. – С. 91–94.
4. Білик Н. М. Порівняльна оцінка показників гемостазу при лікуванні ретрохоріальних та ретроплацентарних гематом в I–II триместрах вагітності / Н. М. Білик // *Педіатрія, акушерство та гінекологія*. – 2006. – №1. – С. 89–91.
5. Борисова Є. А. Профілактика невиношування вагітності у жінок із запальними захворюваннями урогенітального тракту / О. О. Борисова, Н. І. Дуглас // *Якутський медичний журнал*. – 2006. – Т. 16, №4. – С. 47–50.
6. Вакуленко Г. А. Шляхи оптимізації лікування патології шийки матки / Г. А. Вакуленко, Є. В. Коханевич, А. В. Базарна // *Здоров'я жінки*. – 2003. – Т. 13, №1.
7. Вдовиченко Ю. П. Застосування Протефлазиду для профілактики акушерських та перинатальних ускладнень у жінок із герпетичною інфекцією / Ю. П. Вдовиченко, П. М. Баскаков, І. С. Глазков // *Науково-практичний журнал для акушерів-гінекологів «З турботою про жінку»*. – 2011. – №5. – С. 35–37.
8. Внутрішньоутробні інфекції групи TORCH (токсоплазмоз, краснуха, цитомегалія, герпетична інфекція). Епідеміологія, клініка, діагностика, лікування, диспансеризація, профілактика. [Методичні рекомендації МОЗ України.] – К., 2008. – 31 с.
9. Вовк І. Б. Використання флавоноїдів у комплексному лікуванні жінок із запальними захворюваннями геніталій вірусно-бактеріальної етіології / І. Б. Вовк, О. О. Ревенько, О. І. Данилюк // *Здоров'я жінки*. – 2002. – Т. 12, №4. – С. 43–45.
10. Возіанова Ж. І. Грип / Ж. І. Возіанова, А. М. Печінка // *Лікування та діагностика*. – 2002. – №2. – С. 23–30.
11. Волошина Н. М. Особливості перебігу папіломавірусної інфекції у вагітних / Н. М. Волошина // *Репродуктивне здоров'я жінки*. – 2006. – Т. 28, №3. – С. 82–85.
12. Генік Н. І. Протефлазид у комплексній терапії ускладнень вагітності у жінок з хламідійною інфекцією / Н. І. Генік // *Репродуктивне здоров'я жінки*. – 2002. – Т. 12, №3. – С. 13–16.
13. Грищенко О. В. Комплексна імунomodуюча терапія папіломавірусної інфекції у вагітних / О. В. Грищенко, В. В. Бобрицька, С. А. Пак // *Збірник наукових праць Асоціації акушерів-гінекологів України*. – К.: Інтермед., 2005. – С. 112–115.
14. Діагностика та лікування інфекцій статевих органів у вагітних. [Методичні рекомендації МОЗ України.] – К., 2005. – 31 с.
15. Дуда О. К. Грип та інші гострі респіраторні вірусні інфекції у вагітних: особливості лікування / О. К. Дуда, В. О. Бойко, Ю. О. Жигарьова // *Здоров'я жінки*. – 2012. – Т. 67, №1. – С. 208–212.
16. Засць-Кахновець О. І. Профілактика невиношування у жінок із запальними захворюваннями урогенітального тракту / О. І. Засць-Кахновець // *Репродуктивне здоров'я жінки* – 2005. – №1. – С. 53–56.

17. Міцода Р. М. Патогенетичні особливості, прогнозування, профілактика та лікування акушерських ускладнень у жінок, які страждали або страждають на вірусні гепатити: автореф. дис. на присвоєння наук. ступеня канд. мед. наук: спец. 14.01.01 «Акушерство та гінекологія» / Міцода Р. М. – К., 2007. – 36 с.
18. Міцода Р. М. Післяпологовий період у жінок із реплікацією збудників вірусних гепатитів під час гестації та корекції ускладнень Протефлазідом / Р. М. Міцода // Репродуктивне здоров'я. – 2006. – Т. 25, №1. – С. 154–156.
19. Нагорна В. Ф., Ніколаєва С. В. Механізм імуноспрямованої дії Протефлазиду під час вагітності у пацієнток із невиношуванням вагітності вірусної етіології / В. Ф. Нагорна, С. В. Ніколаєва // Здоров'я жінки. – 2007. – Т. 29, №1. – С. 111–113.
20. Островська О. М. Деякі аспекти застосування препарату Протефлазид у комплексному лікуванні загрози переривання вагітності у жінок з хламідійною інфекцією / О. М. Островська // Репродуктивне здоров'я жінки. – 2002. – Т. 11, №2. – С. 9–12.
21. Пилипенко О. М. Відновлення репродуктивної функції при невиношуванні вагітності / О. М. Пилипенко, А. А. Залізна, Я. Ю. Борисова та співавт. // Імунологія та алергологія. – 2002. – №3. – С. 63.
22. Резніченко Н. А. Діагностика та лікування генітального кандидозу, викликаного *C. albicans* у вагітних / Н. А. Резніченко, О. С. Прилуцький, Е. А. Майлян та ін. // Імунологія та алергологія. – 2004. – №2. – С. 6.
23. Резніченко Г. І., Резніченко Ю. І., Павлюченко Н. П. та ін. Деякі аспекти профілактики внутрішньоутробної інфекції / Г. І. Резніченко, Ю. І. Резніченко, Н. П. Павлюченко та ін. // Збірник наукових праць Асоціації акушерів-гінекологів України. – К.: Інтермед., 2005. – С. 358–361.
24. Сергієнко С. М. Вплив Протефлазиду на активність імунотоксичних та аутоімунних реакцій у вагітних із синдромом затримки внутрішньоутробного розвитку плода / С. М. Сергієнко // Проблеми екологічної та медичної генетики і клінічної імунології. Збірник наукових праць. – Київ-Луганськ – Харків., 2003. – С. 356–363.
25. Сімрок В. В. Ефективність Протефлазиду у профілактиці вірусних інфекцій у вагітних / В. В. Сімрок, О. В. Гордієнко // Збірник наукових праць «Проблеми екологічної та медичної генетики та клінічної імунології». – Київ – Луганськ – Харків, 2003. – С. 347–356.
26. Структура причин невиношування вагітності на Одещині / В. Ф. Нагорна, С. В. Ніколаєва, С. П. Посохова, Л. І. Гуменюк // Репродуктивне здоров'я жінки. – 2006. – №2. – С. 58–62.
27. Танько О. П. Імуномодельююча терапія вальвовірусів у вагітних / О. П. Танько, Ю. І. Скорбач // Репродуктивне здоров'я жінки. – 2007. – Т. 30, №1. – С. 90–92.
28. Експериментальне вивчення протівірусної ефективності препарату Протефлазид® щодо збудника грипу А (H1N1) на моделі грипової пневмонії у тварин: [звіт]. – С.-Петербург, 2010. – 36 с.

Reference: V. V. Kaminsky, M. N. Shalko, O. Y. Hrynevych. *PROTEFLAZID® in pregnant women: systematic review of observing of postregistration safety and efficacy* // *Woman health*. – 2014. – No. 6 (92). – pp. 160–164.

6.2. PROTEFLAZID®: A META-ANALYSIS OF CLINICAL STUDIES TO ASSESS THE EFFICACY AND SAFETY OF PREGNANT

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Abstract

The article presents results of a meta-analysis of clinical observations and long-term results of the use of «PROTEFLAZID®» in obstetrician practice. A meta-analysis of 24 cohorts post-marketing clinical observations, which covered 1,028 pregnant women. It proved high efficiency and safety of the drug in the treatment and prevention of viral and viral-bacterial infections during pregnancy, as well as preventing the development of perinatal complications.

Key words: PROTEFLAZID®, pregnant women, the meta-analysis, viral infections, clinical studies.

Introduction

Prevention and treatment of the viral and bacterial and viral infections in the obstetric and gynecological practice is of special importance due to their prevalence and possible consequences to the mother and child.

Treatment during pregnancy is associated with certain difficulties as it is impossible to administer many classes of medicinal products that are prohibited or not recommended for use in this period. At the same time, the medicinal products that exert both antiviral and immunomodulatory activity and are allowed to be used during gestation can not only increase the treatment efficacy of certain diseases caused by viruses but also, due to it, decrease the incidence rate of the obstetric and perinatal complications of pregnancy.

PROTEFLAZID® is one if such medicinal products; it is original, national, herbal product containing the flavonoid glycosides obtained from *Deschampsia caespitola* L. and *Calamagrostis epigeios* L. grasses with the oxygen-containing heterocycle composing the basis of their molecules.

The publications specified in the reference list were used for meta-analysis of the results of clinical studies of efficacy and safety of medicinal product PROTEFLAZID® used in the pregnant women.

Material and methods

Three independent experts – obstetricians and gynecologists – took part in the selection of publications to ensure the quality of the analysis. Based on the selected scientific publications pertinent to the studies, the identifier of publications was created including the main author, year of publication and number of pregnant women who received PROTEFLAZID® (Table 1).

Table 1. Pregnant women who received medicinal product PROTEFLAZID® (identifier of publications)

Author, year of publication	Number of pregnant women	Author, year of publication	Number of pregnant women
Mitsoda R. M., 2013	42	Nagornaia V. F., 2007	20
Serhienko S. M., 2003	48	Voloshyna N. N., 2007	136
Reznichenko H. I., 2005	130	Ostrowska O. N., 2002	42
Reznichenko N. A., 2004	18	Mitsoda R. M., 2006	35
Bilyk N. M., 2005	20	Beniuk V. O., 2012	30
Bilyk N. M., 2006	20	Vdovychenko Yu. P., 2011	30
Mintser O. P., Mitsoda R. M., 2006	49	Henyk N. I., 2002	30
Mitsoda R. M., 2007	42	Borysova E. A., 2006	30
Tanko O. P., 2007	30	Zaiets-Kakhnovets O. I., 2005	50
Azimova E. I., 2011	30	Mitsoda R. M., 2005	26
Hlazkov I. S., 2003	100	Tanko O. P., 2006	30
Hryshchenko O. V., 2005	21	Simrok V. V., 2003	19
Total		1 028	

The cohort, comparative, post-marketing, retrospective studies were selected to perform the meta-analysis.

Based on the evaluations of the efficacy and safety of medicinal product PROTEFLAZID® used in pregnant women that were carried out in individual studies, we performed the summarization and quantitative evaluation of the clinical effects in the form of the meta-analysis with an assessment of the statistical significance of the results.

In total, 24 studies (1028 pregnant women from the study group) were included into the meta-analysis. Considering the variety of the clinical efficacy criteria in individual studies, we performed the analysis by each criterion. The analysis did not include variables used in single studies (1–2 times). Such results are provided in the publications themselves and are not subject to summarization currently (till the new study results are available).

The statistical analysis was performed using the licensed version of Stata 12 program.

Study results and their discussion

Risk of preterm delivery. The results of 10 studies were included into analysis of preterm delivery risk: study group (PROTEFLAZID®) – 399 pregnant women, reference group – 444 pregnant women. The obtained results indicate the insignificant heterogeneity (typicality) of the results of individual studies – heterogeneity factors $P^2 = 0.0\%$, $p = 0.860$. Decrease of preterm delivery probability secondary to PROTEFLAZID® administration is observed in all provided studies. The summary estimate of all results allows making a conclusion about the significant

decrease of preterm delivery probability (by 60%) when the medicinal product PROTEFLAZID® is added to the treatment regimen – integral estimate of the odds ratio (OR) = 0.402 (95% CI = 0.17–0.34); $z = 4.74$; $p = 0.0001$ (Table 2).

Table 2. Meta-analysis of preterm delivery risk secondary to administration of medicinal product PROTEFLAZID® (evaluation of odds ratio [OR])

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Henyk N. I., 2002	0.683	0.162	2.868	5.29
Hlazkov I. S., 2003	0.574	0.297	1.112	26.31
Simrok V. V., 2003	0.138	0.006	3.119	3.03
Zaiets-Kakhnovets O. I., 2005	0.275	0.082	0.924	12.36
Tanko O. P., 2006	0.115	0.006	2.173	4.95
Borysova E. A., 2006	0.318	0.096	1.049	10.75
Mitsoda R. M., 2007	0.744	0.175	3.151	4.94
Vdovychenko Yu. P., 2011	0.286	0.091	0.899	12.54
Beniuk V. O., 2012	0.286	0.091	0.899	12.54
Mitsoda R. M., 2013	0.385	0.092	1.603	7.28
Overall (OR)	0.402	0.275	0.586	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 0.0\%$; $p = 0.860$.

The statistical significance of the overall estimate of meta-analysis model (odds ratio): OR = 0.402; $z = 4.74$; $p = 0.0001$. The graphical results are provided in Figure 1.

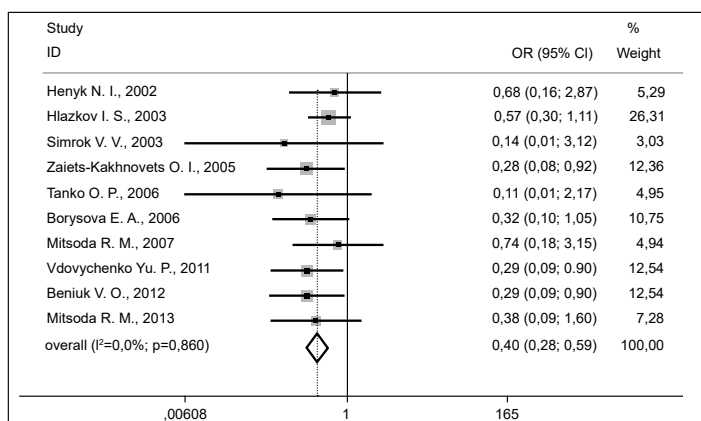


Figure 1. Meta-analysis of preterm delivery risk (evaluation of odds ratio [OR]) in pregnant women secondary to administration of medicinal product PROTEFLAZID®

There is a decreased risk of the preterm delivery secondary to PROTEFLAZID® administration in all studies; however, the statistically significant estimate ($p < 0.05$) is common only for the studies where the confidence interval does not intersect 1 (risk decrease from minimal to significant). Integral estimate of 10 studies confirms the hypothesis of efficacy of medicinal product PROTEFLAZID® in decreasing the odds of preterm delivery.

Risk of threatened miscarriage and premature miscarriage. The analysis of the risk of threatened miscarriage and above all premature miscarriage secondary to administration of medicinal product PROTEFLAZID® included 323 pregnant women from the study group (PROTEFLAZID®) and 358 pregnant women from the control group (10 studies) (Table 3).

Table 3. Meta-analysis of threatened miscarriage and premature miscarriage risk secondary to administration of medicinal product PROTEFLAZID® (evaluation of odds ratio [OR])

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Henyk N. I., 2002	0.117	0.025	0.546	10.33
Hlazkov I. S., 2003	0.471	0.068	3.261	2.33
Simrok V. V., 2003	0.118	0.047	0.293	21.13
Zaiets-Kakhnovets O. I., 2005	0.053	0.006	0.472	7.38
Tanko O. P., 2006	0.274	0.072	1.037	6.61
Borysova E. A., 2006	0.557	0.218	1.424	9.47
Mitsoda R. M., 2007	0.609	0.195	1.897	5.96
Vdovychenko Yu. P., 2011	0.237	0.081	0.693	10.65
Beniuk V. O., 2012	0.237	0.081	0.693	10.65
Mitsoda R. M., 2013	0.197	0.078	0.501	15.48
Overall (OR)	0.240	0.168	0.343	100.00

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 20.8\%$; $p = 0.251$.

Significantly ($p = 0.0001$) decreased probability of premature miscarriage secondary to administration of medicinal product PROTEFLAZID® (by 76%) was revealed – odds ratio (OR) = 0.240 (95% CI = 0.17–0.34). The presented studies have low heterogeneity estimate: $I^2 = 20.8\%$, $p = 0.251$ (Figure 2).

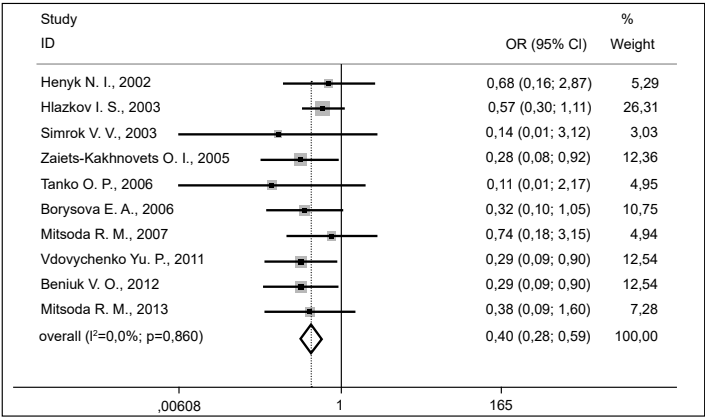


Figure 2. Meta-analysis of premature miscarriage (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

The statistical significance of the overall estimate of meta-analysis model (odds ratio): OR = 0.483; $z = 3.5$; $p = 0.001$.

Risk of disordered labor. The analysis of the risk of the disordered labor secondary to administration of the medicinal product PROTEFLAZID® included 312 pregnant women from the study group (PROTEFLAZID®) and 369 pregnant women from the control group (8 studies) (Table 4).

Table 4. Meta-analysis of disordered labor risk (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Henyk N. I., 2002	0.438	0.128	1.495	12.36
Simrok V. V., 2003	0.471	0.068	3.261	4.39
Hlazkov I. S., 2003	0.683	0.288	1.619	18.43
Mitsoda R. M., 2006	0.461	0.198	1.072	22.87
Tanko O. P., 2006	0.893	0.256	3.113	7.68
Vdovychenko Yu. P., 2011	0.306	0.072	1.291	10.53
Beniuk V. O., 2012	0.306	0.072	1.291	10.53
Mitsoda R. M., 2013	0.337	0.096	1.177	12.23
Overall (OR)	0.483	0.322	0.727	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 0.0\%$; $p = 0.913$.

The statistical significance of the overall estimate of meta-analysis model (odds ratio): OR = 0.483; $z = 3.5$; $p = 0.001$.

Results of individual studies do not allow making a conclusion about the statistically significant decrease of the abnormal labor risk but the summarized results indicate the statistically significant ($p = 0.001$) decrease of the abnormal labor probability secondary to administration of the medicinal product PROTEFLAZID® (by 52%) – odds ratio (OR) = 0.483 (95% CI = 0.32–0.73). The high typicality of the study results can also be noted – model heterogeneity factor: $I^2 = 0.0\%$, $p = 0.913$. The graphical result is provided in Figure 3.

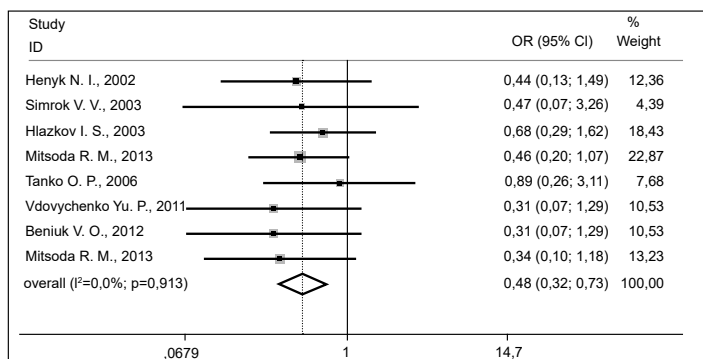


Figure 3. Meta-analysis of abnormal labor risk (evaluation of odds ratio [OR]) in pregnant women secondary to administration of medicinal product PROTEFLAZID®

Risk of fetoplacental insufficiency. Analysis of fetoplacental insufficiency included 232 patients who received the medicinal product PROTEFLAZID® and 252 patients from the control group (5 studies) (Table 5).

Table 5. Meta-analysis of fetoplacental insufficiency risk (evaluation of odds ratio [OR]) in pregnant women secondary to administration of medicinal product PROTEFLAZID®

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Henyk N. I., 2002	0.438	0.128	1.495	18.80
Hlazkov I. S., 2003	0.471	0.068	3.261	6.68
Vdovychenko Yu. P., 2011	0.683	0.288	1.619	28.04
Beniuk V. O., 2012	0.461	1.198	1.072	34.80
Mitsoda R. M., 2013	0.893	0.256	3.113	11.68
Overall (OR)	0.570	0.353	0.921	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 0.0\%$; $p = 0.891$.

The statistical significance of the overall estimate of meta-analysis model (odds ratio): OR = 0.570; $z = 2.30$; $p = 0.022$.

Significantly decreased odds of the fetoplacental insufficiency secondary to administration of medicinal product PROTEFLAZID® (by 43%) was revealed – OR = 0.570 (95% CI = 0.353–0.921), $p = 0.022$. Decreased probability of this pathology is also found in the individual studies, but the statistically significant estimate is formed only when the results are summarized. The graphical results are provided in Figure 4

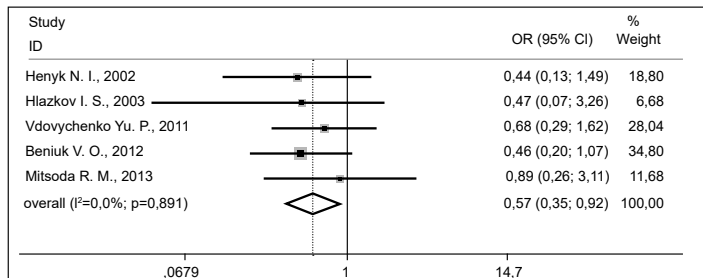


Figure 4. Meta-analysis of fetoplacental insufficiency risk (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

Risk of preeclampsia. Analysis of preeclampsia risk secondary to administration of medicinal product PROTEFLAZID® included 198 pregnant women from the study group (PROTEFLAZID®) and 212 pregnant women from the control group (5 studies) (Table 6). Results of not all individual studies allow making a conclusion about the statistically significant decrease of the preeclampsia risk.

Results of not all individual studies allow making a conclusion about the statistically significant decrease of preeclampsia risk secondary to administration of medicinal product PROTEFLAZID®, but the summarized results indicate the formation of the statistically significant changes ($p = 0.05$) of the decrease of preeclampsia probability when the medicinal product PROTEFLAZID® is used (by 43%) – odds ratio (OR) = 0.568 (95% CI = 0.322–1.00). The high typicality of the study results is also formed – model heterogeneity factor: $I^2 = 0.0\%$, $p = 0.418$.

Table 6. Meta-analysis of preeclampsia risk (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Hlazkov I. S., 2003	0.438	0.204	1.142	47.81
Tanko O. P., 2006	4.529	0.177	115.594	1.19
Azimova E. I., 2011	0.818	0.281	2.382	23.03
Mitsoda R. M., 2013	0.337	0.096	1.177	27.96
Overall (OR)	0.568	0.322	1.001	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 0.0\%$; $p = 0.418$.

The statistical significance of the overall estimate of meta-analysis model (odds ratio): OR = 0.568; $z = 1.96$; $p = 0.050$. The graphical results are provided in Figure 5.

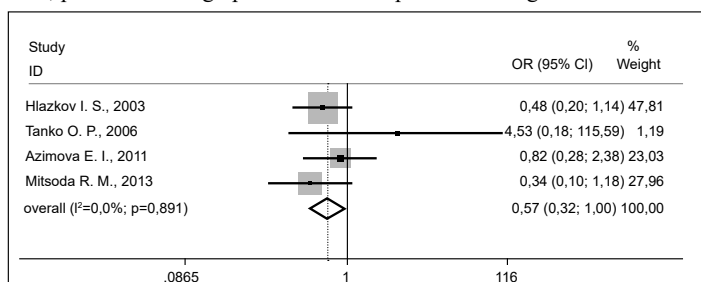


Figure 5. Meta-analysis of preeclampsia risk (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

Risk of anemia of pregnancy. Analysis of anemia of pregnancy risk included 295 patients who received the medicinal product PROTEFLAZID® and 279 patients from the control group (4 studies) (Table 7). Significant decrease of odds of anemia of pregnancy secondary to administration of medicinal product PROTEFLAZID® (by 47%) was confirmed – OR = 0.526 (95% CI = 0.365–0.781), $p = 0.001$. Decreased probability of this pathology is revealed in all presented studies – at the end of the study, but the statistically significant estimate is formed when the results are summarized and level of heterogeneity estimates of individual studies is low.

Table 7. Meta-analysis of anemia of pregnancy risk (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Hlazkov I. S., 2003	0.483	0.275	0.848	47.65
Tanko O. P., 2006	0.522	0.245	1.111	24.90
Azimova E. I., 2011	0.713	0.292	1.743	15.90
Mitsoda R. M., 2013	0.526	0.171	1.620	11.55
Overall (OR)	0.526	0.365	0.781	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 0.0\%$; $p = 0.912$.

The statistical significance of the overall estimate of meta-analysis model (odds ratio): OR = 0.526; $z = 3.24$; $p = 0.001$. The graphical results are provided in Figure 6.

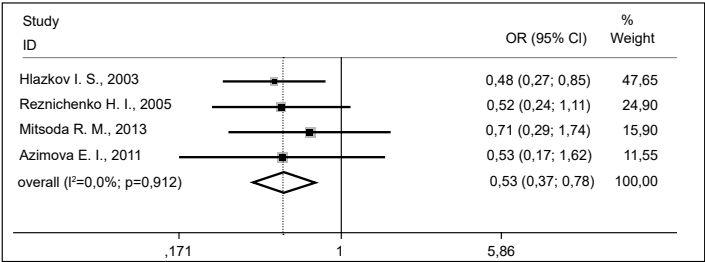


Figure 6. Meta-analysis of anemia of pregnancy risk (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

Risk of infection generalization in pregnancy. This meta-analysis includes the results of 2 studies by three parameters: 2 – by variables of infection generalization and 1 – by variable of rate of positive reactions to TORCH infection. Analysis included 279 patients who received the medicinal product PROTEFLAZID® and 187 pregnant women from the control group (Table 8).

Table 8. Meta-analysis of risk of infection generalization in pregnancy (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Simrok V. V., 2003	0.483	0.275	0.848	47.65
Reznichenko H. I., 2005 (1)	0.522	0.245	1.111	24.90
Reznichenko H. I., 2005 (2)	0.713	0.292	1.743	15.90
Overall (OR)	0.658	0.393	1.102	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 0.0\%$; $p = 0.6532$.

The statistical significance of the overall estimate of meta-analysis model (odds ratio): OR = 0.658; $z = 1.59$; $p = 0.111$.

The trend towards more favorable results is noted, statistically significant decrease of risk of the infection generalization in pregnancy secondary to administration of medicinal product PROTEFLAZID® in the study group – OR = 0.658 ($p = 0.111$) (Figure 7).

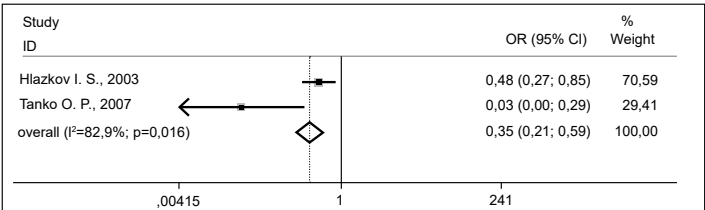


Figure 7. Meta-analysis of risk of infection generalization in pregnancy (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

Risk of vulvovaginitis in pregnancy. Analysis of risk of vulvovaginitis in pregnancy secondary to administration of medicinal product PROTEFLAZID® included 130 pregnant women from the study group (PROTEFLAZID®) and 130 pregnant women from the control group (2 studies) (Table 9).

Table 9. Meta-analysis of risk of vulvovaginitis in pregnancy (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Hlazkov I. S., 2003	0.483	0.275	0.848	70.59
Tanko O. P., 2007	0.034	0.004	0.287	29.41
Overall (OR)	0.351	0.209	0.589	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 82.9\%$; $p = 0.016$.

The results of the presented studies allow making a conclusion about the statistically significant decrease of the vulvovaginitis risk secondary to administration of medicinal product PROTEFLAZID®, which is also confirmed by the summarization of results and indicates the formation of the statistically significant estimates ($p = 0.0001$) of the decrease of vulvovaginitis probability when the medicinal product PROTEFLAZID® is used (by 65%) – odds ratio (OR) = 0.351 (95% CI = 0.21–0.59). Heterogeneity of study results by this criterion is formed – model heterogeneity factor: $I^2 = 82.9\%$; $p = 0.016$ (Figure 8).

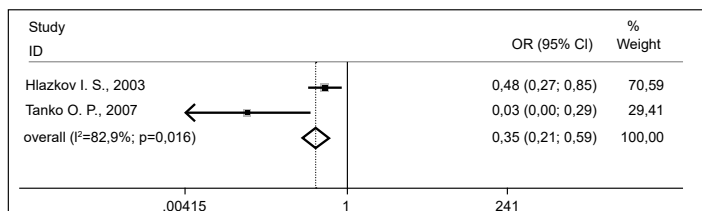


Figure 8. Meta-analysis of risk of vulvovaginitis in pregnancy (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

The statistical significance of the overall estimate of meta-analysis model (evaluation of odds ratio): OR = 0.351; $z = 3.97$; $p = 0.0001$.

Risk of neonatal asphyxia. Analysis of neonatal asphyxia risk secondary to administration of medicinal product PROTEFLAZID® during pregnancy included 8 studies: 454 pregnant women from the study group (PROTEFLAZID®) and 426 pregnant women from the control group (Table 10).

Table 10. Meta-analysis of neonatal asphyxia risk (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Hlazkov I. S., 2003	0.527	0.267	1.040	23.50
Zaiets-Kakhnovets O. I., 2005	0.444	0.140	1.411	9.10
Reznichenko H. I., 2005	0.616	0.317	1.194	22.14
Borysova E. A., 2006	0.154	0.007	3.365	2.71
Mitsoda R. M., 2007	1.354	0.525	3.494	7.33
Vdovychenko Yu. P., 2011	0.398	0.131	1.210	10.08
Beniuk V. O., 2012	0.327	0.104	1.032	10.52
Mitsoda R. M., 2013	0.378	0.149	0.963	14.64
Overall (OR)	0.534	0.385	0.741	100.00

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 0.0\%$; $p = 0.529$.

Results of each presented study allow making a conclusion about the trend towards decreased risk of the neonatal asphyxia secondary to administration of medicinal product PROTEFLAZID®. Summarization of results forms the statistically significant estimates ($p = 0.0001$) of decrease of the neonatal asphyxia probability by 57% when the medicinal product PROTEFLAZID® is received – odds ratio (OR) = 0.534 (95% CI = 0.385–0.741), $p = 0.0001$.

The statistical significance of the overall estimate of meta-analysis model (evaluation of odds ratio): OR = 0.534; $z = 3.76$; $p = 0.0001$. The graphical results are provided in Figure 9.

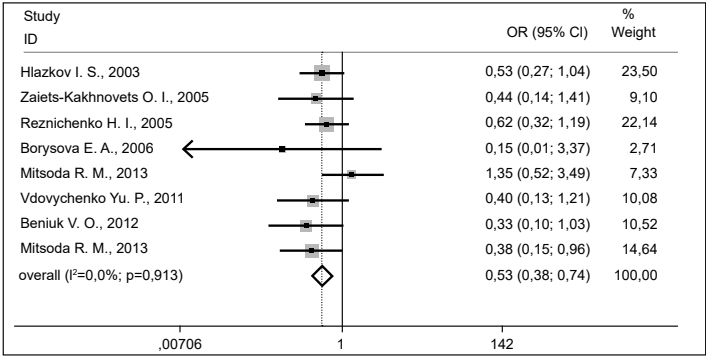


Figure 9. Meta-analysis of neonatal asphyxia risk (evaluation of odds ratio OR) secondary to administration of medicinal product PROTEFLAZID®

Risk of neonatal encephalopathy. Meta-analysis of the neonatal encephalopathy risk included 222 patients from the study group (PROTEFLAZID®) and 239 pregnant women from the control group (Table 11).

Table 11. Meta-analysis of neonatal encephalopathy risk (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Zaiets-Kakhnovets O. I., 2005	0.329	0.132	0.824	30.42
Borysova E. A., 2006	0.286	0.065	1.253	12.75
Mitsoda R. M., 2007	0.891	0.393	2.024	22.54
Overall (OR)	0.513	0.393	0.806	100.00

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 7.5\%$; $p = 0.355$.

Secondary to the low heterogeneity of the results ($I^2 = 7.5\%$), summarization of data of 4 studies allows making a conclusion about the statistically significant decrease of neonatal encephalopathy risk (by 48.7%) secondary to administration of medicinal product PROTEFLAZID® during pregnancy – odds ratio (OR) = 0.513 (95% CI = 0.393–0.806), $p = 0.004$.

The statistical significance of the overall estimate of meta-analysis model (evaluation of odds ratio): OR = 0.513; $z = 2.9$; $p = 0.004$. The graphical results are provided in Figure 10.

To analyze the changes of the quantitative variables and perform their comparative assessment, we used the “effect size” parameter; this parameter is also referred to as “standardized mean difference” (SMD).

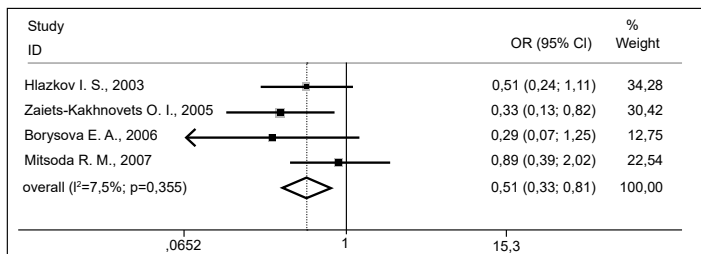


Figure 10. Meta-analysis of neonatal encephalopathy risk (evaluation of odds ratio [OR]) secondary to administration of medicinal product PROTEFLAZID®

The standardized mean difference is used as the summary statistic in meta-analysis when all studies evaluate the same outcome but the measurements are presented in the different units or have different absolute size of changes and variability of parameters. In this case, it is necessary to standardize the study outcomes using the single scale before they can be pooled.

The standardized mean difference reflects the effect size obtained as a result of the intervention (administration of medicinal product) in each study taking into consideration the variability of the outcomes in each group. The estimate is as follows: how many times does the standardized effect size (parameter change) in the study group exceed the similar parameter in the reference group.

The estimation principle – “normalization of parameter” – is used in all presented below studies. The baseline abnormal levels of parameters are recovered to the “normal level” (reference value). Insignificant changes of the parameter do not lead to its normalization. And on the contrary, the marked changes in the direction of recovery of normal values of the study parameter are the confirmation of the intervention efficacy (administration of medicinal product).

Effect size in the study group (PROTEFLAZID®) versus control group by CD3 parameter. The results provided by CD3 parameter indicate that the findings of 4 studies (120 patients from the study group [PROTEFLAZID®] and 140 pregnant women from the control group) demonstrate the statistically significant superiority of effect size (by 3.3 times) in the group of pregnant women secondary to administration of medicinal product PROTEFLAZID® compared with the control group – SMD = 3.33; (95% CI = 2.94–3.73); $p = 0.0001$ (Table 12). It confirms the more intensive recovery of the immunological measures by CD3 parameter.

Table 12. Meta-analysis of effect size comparison (standardized mean difference) in the study group (PROTEFLAZID®) versus control group by CD3 parameter

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Azimova E. I., 2011	1.855	1.247	2.464	41.28
Nagorna V. F., 2007	8.273	6.312	10.233	3.98
Nagorna V. F., 2007	10.182	8.262	12.103	4.41
Henyk M. I., 2002	3.160	2.489	3.832	33.89
Tanko O. P., 2007	4.459	3.503	5.415	16.71
Overall (SMD)	3.333	2.942	3.723	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 96.1\%$; $p = 0.0001$

Overall estimate of effect exceeding (effect size) in meta-analysis model: $SMD = 3.333$; $z = 16.7$; $p = 0.0001$. The graphical results are provided in Figure 11.

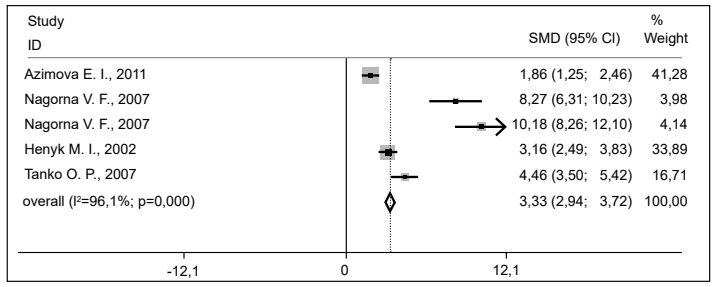


Figure 11. Meta-analysis of effect size comparison (standardized mean difference) in the study group (PROTEFLAZID®) versus control group by CD3 parameter

Effect size in the study group (PROTEFLAZID®) versus control group by CD4 parameter.
The results provided by CD4 parameter indicate that the findings of 6 studies (160 patients from the study group [PROTEFLAZID®] and 170 pregnant women from the control group) demonstrate the statistically significant superiority of effect size (by 2.88 times) in the group of pregnant women secondary to administration of medicinal product PROTEFLAZID® compared with the control group – $SMD = 2.88$; (95% CI = 2.55–3.20); $p = 0.0001$ (Table 13).

Table 13. Meta-analysis of effect size comparison (standardized mean difference) in the study group (PROTEFLAZID®) versus control group by CD4 parameter

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Azimova E. I., 2011	2.057	1.428	2.687	26.35
Nagorna V. F., 2007	1.872	1.123	2.621	18.60
Ostrovskaya O. N., 2002	3.671	2.639	4.703	9.80
Nagorna V. F., 2007	2.454	1.705	3.203	18.61
Henyk N. I., 2002	4.000	3.226	4.774	17.43
Tanko O. P., 2007	5.145	4.081	6.209	9.22
Overall (SMD)	3.333	2.942	3.723	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 88.2\%$; $p = 0.0001$.

It confirms the efficacy of medicinal product PROTEFLAZID® in recovery of the immunological measures by CD4 parameter.

Overall estimate of effect exceeding (effect size) in meta-analysis model: $SMD = 2.878$; $z = 17.5$; $p = 0.0001$. The graphical results are provided in Figure 12.

Effect size in the study group (PROTEFLAZID®) versus control group by CD8 parameter.
Decrease and normalization of CD8 parameter was investigated in 4 studies involved the pregnant women with the viral infection. The effect sizes that were heterogeneous in magnitude by CD8 changes were revealed but superiority of the study group over control one is mentioned in all publications of the results (Table 14).

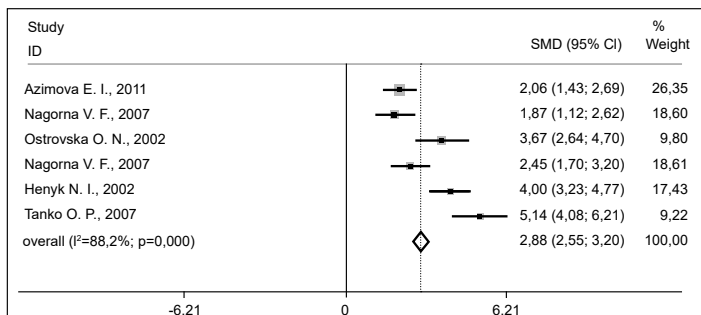


Figure 12. Meta-analysis of effect size comparison (standardized mean difference) in the study group (PROTEFLAZID®) versus control group by CD4 parameter

Table 14. Meta-analysis of effect size comparison (standardized mean difference) in the study group (PROTEFLAZID®) versus control group by CD8 parameter

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Ostrovskaya O. N., 2002	1.97	1.28	2.66	18.22
Nagorna V. F., 2007	1.72	0.99	2.45	16.2
Azimova E. I., 2011	0.62	0.1	1.14	32.21
Tanko O. P., 2007	0.32	-0.19	0.83	33.37
Overall (SMD)	3.333	2.942	3.723	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 85.1\%$; $p = 0.0001$.

The standardized effect size revealed the statistically insignificant difference versus control group only in 1 of 4 studies (Tanko O. P., 2007); $SMD = 0.32$ (0.19–0.83) ($p > 0.05$). Other 3 studies demonstrated the significant superiority of CD8 changes in the group with medicinal product PROTEFLAZID® versus control group: $SMD: 0.62$ – 1.97 .

Overall estimate of effect exceeding (effect size) in meta-analysis model: $SMD = 0.94$; $z = 6.3$; $p = 0.0001$. The graphical results are provided in Figure 13.

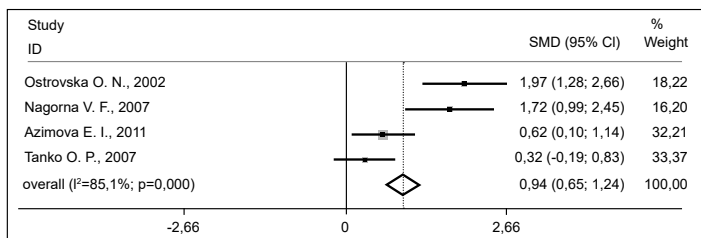


Figure 13. Meta-analysis of superiority of recovery effect size secondary to administration of medicinal product PROTEFLAZID® versus control group by CD8 parameter

Effect size in the study group (PROTEFLAZID®) versus control group by CD19 parameter. The provided results indicate that the findings of 3 studies (80 patients from the study group [PROTEFLAZID®] and 80 pregnant women from the control group) demonstrate the superiority of effect size (by 1.9 times) in the group of pregnant women secondary to administration of medicinal product PROTEFLAZID® compared with the control group – SMD = 1.905; (95% CI = 1.497–2.313); p = 0.0001 (Table 15).

Table 15. Meta-analysis of effect size comparison (standardized mean difference) in the study group (PROTEFLAZID®) versus control group by CD19 parameter

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Azimova E. I., 2011	0.759	0.234	1.284	60.55
Nagorna V. F., 2007	3.762	2.713	4.810	15.15
Tanko O. P., 2007	3.602	2.774	4.430	24.30
Overall (SMD)	1.905	1.497	2.131	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 95.75\%$; $p = 0.0001$.

It indicates the more intensive recovery of the immunological measures. The significant heterogeneity of the results (heterogeneity factor: $I^2 = 95.75\%$) may be explained by different criteria of the study (pathological process severity, different types of infections, follow-up duration, etc.).

Overall estimate of effect exceeding (effect size) in meta-analysis model: SMD = 1.905; $z = 9.15$; $p = 0.0001$. The graphical results are provided in Figure 14.

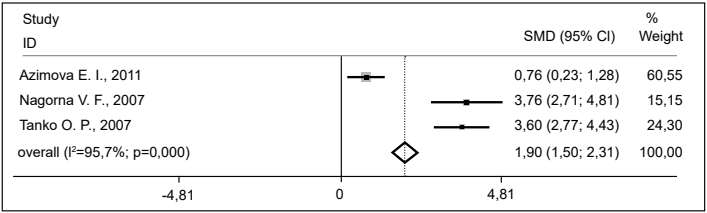


Figure 14. Meta-analysis of effect size superiority in the study group (PROTEFLAZID®) versus control group by CD19 parameter

Effect size in the study group (PROTEFLAZID®) versus control group by IgG parameter. The results provided by IgG parameter indicate that the findings of 3 studies (79 patients from the study group [PROTEFLAZID®] and 95 pregnant women from the control group) demonstrate the statistically significant superiority of effect (by 1.29 times) in the group of pregnant women secondary to administration of medicinal product PROTEFLAZID® versus the control group – SMD = 1.29; (95% CI = 0.94–1.63); p = 0.0001 (Table 16). It confirms the efficacy of medicinal product PROTEFLAZID® in recovery of the immunological measures by IgG parameter.

Overall estimate of effect exceeding (effect size) in meta-analysis model: SMD = 1.286; $z = 7.26$; $p = 0.0001$. The graphical results are provided in Figure 15.

Table 16. Meta-analysis of effect size comparison (standardized mean difference) in the study group (PROTEFLAZID®) versus control group by IgG parameter

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Azimova E. I., 2011	0.759	0.234	1.284	60.55
Nagorna V. F., 2007	3.762	2.713	4.810	15.15
Tanko O. P., 2007	3.602	2.774	4.430	24.30
Overall (SMD)	1.905	1.497	2.131	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 93.7\%$; $p = 0.0001$

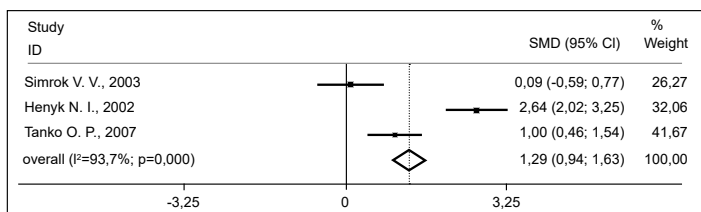


Figure 15. Meta-analysis of effect size superiority in the study group secondary to administration of medicinal product PROTEFLAZID® versus control group by IgG parameter

Effect size in the study group (PROTEFLAZID®) versus control group by CIC (circulating immune complexes) parameter. The results provided by CIC parameter indicate that the findings of 3 studies (90 patients from the study group [PROTEFLAZID®] and 112 pregnant women from the control group) demonstrate the statistically significant superiority of effect size (by 1.55 times) in the group of pregnant women secondary to administration of medicinal product PROTEFLAZID® compared with the control group – SMD = 1.55; (95% CI = 1.2–1.9); $p = 0.0001$ (Table 17).

Table 17. Meta-analysis of effect size comparison (standardized mean difference) in the study group (PROTEFLAZID®) versus control group by CIC parameter

Study	Odds ratio (OR)	95% confidence interval		Weight (%)
Simrok V. V., 2003	0.816	0.111	1.522	24.57
Nagorna V. F., 2007	0.012	–0.607	0.632	31.87
Henyk N. I., 2002	3.880	3.121	4.638	21.27
Serhienko S. M., 2003	2.316	1.575	3.057	22.29
Overall (SMD)	1.55	1.196	1.896	100.0

Note: Assessment of meta-analysis model heterogeneity: $I^2 = 68.1\%$; $p = 0.0001$

It confirms the efficacy of medicinal product PROTEFLAZID® in recovery of immunological measures by CIC parameter.

Overall estimate of effect exceeding (effect size) in meta-analysis model: SMD = 1.55; $z = 8.7$; $p = 0.0001$. The graphical results are provided in Figure 16.

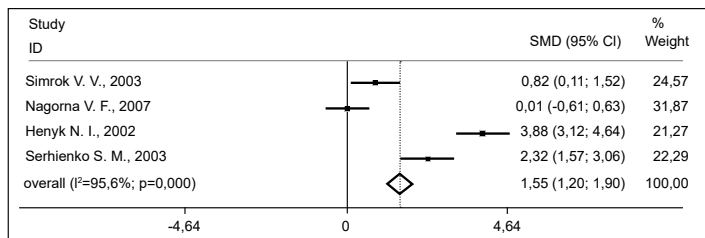


Figure 16. Meta-analysis of effect size superiority in the study group secondary to administration of medicinal product PROTEFLAZID® versus control group by CIC parameter

Many other immunological measures that in the individual studies also demonstrate the trend or statistically significant superiority of recovery changes secondary to administration of medicinal product PROTEFLAZID® versus control group were not included into the analysis due to the methodological limitations of meta-analysis procedure – mainly due to the limited number of such results or the difficulty to compare the data.

Conclusions

Meta-analysis of the cohort, comparative, randomized, post-marketing, retrospective clinical studies involved pregnant women was performed to evaluate the efficacy and safety of medicinal product PROTEFLAZID® used in treatment of the viral and bacterial and viral infections. The group of patients who received the medicinal product PROTEFLAZID® comprised 1 028 women.

The comparative analysis confirmed the superior efficacy of medicinal product PROTEFLAZID® by the following clinical criteria: risk of preterm delivery, risk of threatened miscarriage and premature miscarriage, risk of abnormal labor, risk of fetoplacental insufficiency, risk of preeclampsia, risk of anemia of pregnancy, risk of infection generalization, risk of unfavorable course of vulvovaginitis, risk of neonatal asphyxia and encephalopathy.

Analysis of the quantitative variables demonstrated the effect size superiority by the following parameters: CD3, CD4, CD8, CD19, IgG, CIC in the group of pregnant women who received the medicinal product PROTEFLAZID® compared with the control group indicating the efficacy of medicinal product PROTEFLAZID® in recovery of the immune system parameters in the viral and bacterial and viral infections.

No side effects were observed. Also no clinically significant laboratory abnormalities were recorded secondary to administration of medicinal product PROTEFLAZID® indicating its efficacy and safety in the treatment and prevention of the viral and bacterial and viral infections in the pregnant women.

References:

1. Міцода Р. М. Система акушерської допомоги жінкам, які страждали або страждають на вірусні гепатити [Матеріали конференції «Внутрішня патологія в акушерстві та гінекології»] // Медичні аспекти здоров'я жінки. Спеціальний випуск. Інфекції в акушерстві та гінекології – 2013. – С. 28–30.

2. Сергієнко С. М. Вплив Протефлазиду на активність імунотоксичних та аутоімунних реакцій у вагітних із синдромом затримки внутрішньоутробного розвитку плода / С. М. Сергієнко // Збірник наукових праць «Проблеми екологічної та медичної генетики і клінічної імунології. – Київ – Луганськ –Харків, 2003. – С. 356–363.
3. Резніченко Г. І. Деякі аспекти профілактики внутрішньоутробної інфекції / Резніченко Г. І., Резніченко Ю. Г., Павлюченко Н. П., Резніченко Н. Ю., Спіріна // Збірник наукових праць Асоціації акушерів-гінекологів України. – К.: Інтермед., 2005. – С. 358–361.
4. Резніченко Н. А. Діагностика та лікування генітального кандидозу, викликаного *C. albicans* у вагітних / Н. А. Резніченко, О. С. Прилуцький, Е. А. Майлян та ін. // Імунологія та алергологія. – 2004. – №2. – С. 6.
5. Білик Н. М. Діагностика та лікування ретрохоріальних та ретроплацентарних гематом у жінок з антифосфоліпідним синдромом та вірусною інфекцією / Н. М. Білик // Педіатрія, акушерство та гінекологія. – 2005. – №2. – С. 91–94.
6. Білик Н. М. Порівняльна оцінка показників гемостазу при лікуванні ретрохоріальних та ретроплацентарних гематом у I–II триместрах вагітності / Н. М. Білик // Педіатрія, акушерство та гінекологія. – 2006. – №1. – С. 89–91.
7. Мінцер О. П. До питання оцінки ефективності лікувально-профілактичних заходів / О. П. Мінцер, Р. М. Міцода // Репродуктивне здоров'я жінки. – 2006. – Т. 29. – №4. – С. 68–72.
8. Міцода Р. М. Патогенетичні особливості, прогнозування, профілактика та лікування акушерських ускладнень у жінок, які страждали або страждають на вірусні гепатити: Автореф. дис. ... на присвоєння наук. ступеня канд. мед. наук: спец. 14.01.01 «Акушерство та гінекологія» / Міцода Р. М. – К., 2007. – С. 36.
9. Танько О. П. Імуномодуюча терапія вульвовагінітів у вагітних / О. П. Танько, Ю. І. Скорбач // Репродуктивне здоров'я жінки. – 2007. – Т. 30. – №1. – С. 90–92.
10. Азімова Е. І. До питання профілактики рецидиву генітального герпесу у вагітних з антенатальною загибеллю плода в анамнезі / Е. І. Азімова, С. М. Султанов, Ф. П. Нішанова // Здоров'я жінки. – 2011. – Т. 58. – №2. – С. 238–240.
11. Глазков І. С. Клініко-патогенетичні аспекти прогнозування, профілактики та лікування акушерських та перинатальних ускладнень при герпетичній інфекції / І. С. Глазков // Репродуктивне здоров'я жінки. – 2003. – Т. 16. – №4. – С. 28–30.
12. Грищенко О. В. Комплексна імуномодуюча терапія папіломавірусної інфекції у вагітних / О. В. Грищенко, В. В. Бобрицька, С. А. Пак // Збірник наукових праць Асоціації акушерів-гінекологів України. – К.: Інтермед., 2005. – С. 112–115.
13. Нагорна В. Ф. Механізм імунотоксичної дії Протефлазиду під час вагітності у пацієнток з невиношуванням вагітності вірусної етіології / В. Ф. Нагорна, С. В. Ніколаєва // Здоров'я жінки. – 2007. – Т. 29, №1. – С. 111–113.
14. Волошина Н. М. Моніторинг патології шийки матки у вагітних / Н. М. Волошина, О. Ю. Петрова, Т. П. Кузнєцова, Л. І. Єлізарова, О. С. Кошлякова // Здоров'я жінки. – 2007. – Т. 30, №2. – С. 63–67.
15. Островська О. М. Деякі аспекти застосування препарату Протефлазид® у комплексному лікуванні загрози переривання вагітності у жінок з хламідійною інфекцією / О. М. Островська // Репродуктивне здоров'я жінки – 2002. – Т. 11, №2. – С. 9–12.
16. Міцода Р. М. Післяпологовий період у жінок із реплікацією збудників вірусних гепатитів під час гестації та корекції ускладнень Протефлазидом / Міцода Р. М. // Репродуктивне здоров'я. – 2006. – Т. 25, №1. – С. 154–156.

17. Бенюк В. О. Периконцепційна профілактика та лікування герпесвірусної інфекції у вагітних з метаболічним синдромом / В. О. Бенюк, О. О. Діндар, П. О. Лихолай // *Здоров'я жінки*. – 2012. – Т. 75, №9. – С. 116–120.
18. Вдовиченко Ю. П. Застосування Протефлазиду для профілактики акушерських та перинатальних ускладнень у жінок з герпетичною інфекцією / Ю. П. Вдовиченко, П. М. Баскаків, І. С. Глазков // *Науково-практичний журнал для акушерів-гінекологів «З турботою про жінку»*. – 2011. – №5. – С. 35–37.
19. Генік Н. І. Протефлазид® у комплексній терапії ускладнень вагітності у жінок з хламідійною інфекцією / Н. І. Генік // *Репродуктивне здоров'я жінки*. – 2002. – Т. 12, №3. – С. 13–16.
20. Борисова Є. А. Профілактика невиношення вагітності у жінок з запальними захворюваннями уrogenітального тракту / Є. А. Борисова, Н. І. Дуглас // *Якутський медичний журнал*. – 2006. – Т. 16, №4. – С. 47–50.
21. Засць-Кахновець О. І. Профілактика невиношування у жінок із запальними захворюваннями уrogenітального тракту / О. І. Засць-Кахновець // *Репродуктивне здоров'я жінки* 2005. – №1. – С. 53–56.
22. Міцода Р. М. Пологи у жінок з активною реплікацією збудників вірусних гепатитів під час гестації та корекції ускладнень Протефлазидом / Міцода Р. М. // *Репродуктивне здоров'я жінки* – 2005. – №4. – С. 77–80.
23. Танько О. П. Імуномодельюча терапія вульвовагінітів у вагітних / О. П. Танько, Ю. І. Скорбач // *Репродуктивне здоров'я жінки*. – 2007. – Т. 30, №1. – С. 90–92.
24. Сімрок В. В. Ефективність Протефлазиду у профілактиці вірусних інфекцій у вагітних / В. В. Сімрок, О. В. Гордієнко // *Збірник наукових праць «Проблеми екологічної та медичної генетики і клінічної імунології»*. – Київ – Луганськ – Харків, 2003. – С. 347–356.

Reference: V. A. Benyuk, Y. V Kuvita, O. Y. Hrynevych, O. B. Tonkovid, I. A. Usevich, O. S. Neymark. *PROTEFLAZID® a meta-analysis of clinical studies to assess the efficacy and safety of pregnant // Woman health*. – 2014. – V. 93, No. 7. – pp. 166–176.

6.3. PROTEFLAZID®: OUTCOME ANALYSIS OF CLINICAL TRIALS STUDYING THE IMPACT OF HUMAN HERPESVIRUS INFECTION ON SOME CELLULAR IMMUNITY INDICATORS AND GESTATION COURSE IN WOMEN

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Abstract

An outcome analysis of the clinical trials evaluating the clinical and immunological efficacy of the drug PROTEFLAZID® in the treatment of diseases associated with herpesvirus infection in pregnant women is presented. Objective of the study was to evaluate the effects of PROTEFLAZID® on the cellular component of the immune system and gestation course in women with HHV infection by using meta-analysis and statistical analysis (based on Student's t-test) of the clinical findings. Materials and methods. The analysis comprises 5 scientific sources with the results of controlled clinical trials involving 234 pregnant women during the period from 2003 to 2012. Results. The obtained data prove the efficacy of the preparation PROTEFLAZID® in treating pregnant women with HHV infection. Administration of the drug PROTEFLAZID® in the treatment of herpesvirus infection in pregnant women contributes to the cellular immunity normalization, which in turn has a positive effect on the course and outcome of pregnancy. Conclusions. Therapy with the drug PROTEFLAZID® is etiologically and pathogenetically substantiated, contributes to the normalization of cellular immunity parameters, which, in turn, has a positive effect on the course and outcome of pregnancy.

Key words: PROTEFLAZID®, pregnant women, meta-analysis, statistical analysis, human herpesvirus infection, immunity, clinical trials.

Introduction

One of the highly topical problems of modern obstetrics is viral infections and their effect on the pregnancy, childbirth, postpartum period, foetus and newborn [1,3,4].

Human herpes virus (HHV) infections are among the most common diseases across the globe. From 65 to 90% of the world's population is infected with the herpes simplex virus (HSV). The disease has complex character extrapolating its influence on the realization of women's reproductive function and demographic safety. HHV infections is nowadays associated with a high incidence of complications during pregnancy and adverse perinatal consequences [3,4,10,16].

Herpes infection across pregnancy is a significant risk factor for obstetric and perinatal complications, and the incidence of the latter depends upon the form of herpes infection with an evident predominance of various pathological conditions associated with recurrent infection [6].

Recurrent herpes is an indicator of impaired immunity, which usually decreases across pregnancy. The incidence of chronic persistent (recurrent) viral infections across pregnancy makes up 67.1-77.3%. Exacerbation of herpes infections across pregnancy is caused by: physiological immunosuppression due to reducing the number and activity of T- and B-lymphocytes; increasing the production of corticosteroids, progesterone, estrogen, which have a physiological immunosuppressive effect; refractoriness and hyperactivity of the interferon system; secondary virus-induced immunodeficiency, etc. [3].

For patients with HHV infections, it is typical: a decrease in the production of endogenous interferon, the natural killer activity and antibody-dependent cellular cytotoxicity, a decrease in the absolute number and activity of T-lymphocytes (CD3+ and CD4+ cells), neutrophil granulocytes, and an increase in the number of immune complexes. The identified immunity disorders persist both in the relapse phase and in remission [3].

In case of spreading herpes virus from cell to cell, either upon their contact, or via integrating the virus into a sensitive cell genome, the cell-mediated immune reactions involving cytotoxic killer T lymphocytes come to the forefront. Since viruses are intracellular parasites, the key protection function against them is performed by cellular reactions [9].

Prevention and treatment of viral infections in obstetric and gynaecological practice occupy a special place because of their prevalence and possible maternal and fetal consequences [4].

Treatment of pregnant women has certain difficulties due to the inability to administer medicinal agents of many classes that are prohibited or not recommended for use across pregnancy. At the same time, drugs with antiviral, immunomodulatory activity and simultaneously approved to be administered during the gestation period may not only improve treatment efficacy of certain virus-induced diseases, but also may reduce the incidence of obstetric and perinatal pregnancy complications in such women [10].

A modern drug used in the treatment of viral infections, which shows good results for many years and is used extensively in obstetric practice in treating pregnant women with HHV infections, is Proteflazid® [8].

The formulation of the drug Proteflazid® comprises flavonoid glycosides, which are contained in the wild herbs *Deschampsia caespitosa* L. and *Calamagrostis epigeios* L., and are able to block the DNA polymerase activity in the cells infected with herpes viruses. The drug has no cumulative properties, embryotoxic, teratogenic, carcinogenic effects [7].

In addition to antiviral activity, Proteflazid® has immunotropic properties and a high safety profile.

The flavonoids contained in the drug have a direct antiviral effect on both DNA and RNA viruses, inhibit the DNA and RNA virus replication, both in vitro and in vivo. When conducting preclinical and clinical studies, the antiviral effect of the drug against the viruses of herpes, hepatitis, papillomaviruses, HIV infection, influenza and acute respiratory infections has been identified and proved.

It has been proved that the mechanism of direct antiviral action involves the virus-specific enzyme inhibition – DNA and RNA polymerases, thymidine kinase, reverse transcriptase and neuraminidase [1,4,6].

The drug has immunotropic properties. It protects mucous membranes, normalizing the indices of local immunity (lactoferrin, secretory immunoglobulin A, lysozyme and C₃ component of complement).

It has been established that the drug is an inducer of the endogenous α and γ -interferon synthesis to a physiologically active level, which increases the host's nonspecific resistance to viral and bacterial infections [6,4,10].

A distinctive feature of the drug is that it does not cause the refractoriness of the immune cells with its long-term administration. The drug Proteflazid® has a superior bioavailability, non-toxic, may be actively used for preconception preparation, prevention and treatment of HHV infections in pregnant. Proteflazid® prevents relapses and has prolonged remission potential [1].

Objective

To conduct the metaand statistical analyses (using Student's t-test) of the results of clinical studies evaluating the effect of Proteflazid® on the cellular component of immunity and gestation course in women with HHV infections.

Materials and methods

Data sources and searching

We have conducted the information retrieval of the clinical trials studying the drug Proteflazid® effect on the trend of cellular immunity indicators and gestation course in women with HHV infections on the Internet using information retrieval systems, as well as conference materials in the scientific publications. The scientific articles reflecting the efficacy of Proteflazid® in the treatment of HHV infections in pregnant published during the period from 2003 to 2012.

The list of publications selected for the analysis reflecting the clinical and immunological efficacy of Proteflazid® in treatment of pregnant women with HHV infections is presented in tab.1.

Software

RevMan and SPSS. To ensure the reliability and accuracy of the results, calculations were made by two researchers.

Table 1. List of publications showing the drug Proteflazid® efficacy in treatment of pregnant women with HHV infections

Author, Year	Total number of patients	Scope of drug application	Clinical results
Azimova E.I. , 2011 [2]	60	Recurrent genital herpes in pregnant women with a history of intrauterine foetal death	Improving the immune system function, improving the placental function
Benyuk V.A. et al., 2012 [3]	60	Herpesvirus infection in pregnant women with metabolic syndrome	Improving the immune system function, a considerable reduction in the incidence of perinatal complications

Nikolaeva S.V., 2007 [12]	40	Miscarriage secondary to herpes infection	Improving the immune system function, reducing the viral replication intensity, reducing the antigen load
Simrok V.V. et al. [17]	34	Herpesvirus infection in pregnant women	Improving the clinical picture, positive changes of immunological indicators
Bilyk N.M., 2005 [5]	40	Herpesvirus infection in pregnant women	Improving the clinical picture and gestation course

Inclusion and exclusion criteria

The studies intended for inclusion in the meta-analysis conform to the following criteria:

1. Controlled trials provided the control or control group in studying the clinical efficacy of Proteflazid® in the treatment of pregnant women with HHV infections.
2. Mandatory confirmation of the clinical diagnosis of identified diseases in pregnant women.
3. Published data on the efficacy evaluation of Proteflazid® are complete.

The studies to be excluded from the meta-analysis conform to the following criteria:

1. Uncontrolled clinical studies evaluating the clinical and immunological efficacy of Proteflazid® for the treatment of diseases caused by HHV infections in pregnant women.

2. Doubling of previous publications. If more than one study by the same author of the same case series was published, the latest publication was included in the study.

1. Studies based on incomplete data.
2. No control group.
3. Meta-analyses, letters, reviews or editorials.

In accordance with the inclusion and exclusion criteria, five trials were included in the meta-analysis and seven studies were excluded [11,13-16,18,19].

In the controlled clinical studies that were included in the analysis, 234 pregnant women were involved during the period from 2003 to 2012.

The patients before initiation of treatment and on completing the treatment underwent thorough examination, including generally accepted clinical-laboratory and instrumental tests. To evaluate the immune status, a comprehensive immunological examination was carried out, which included the mature T-lymphocytes and their subpopulations, as well as B-lymphocytes.

The meta-analysis of clinical trials reflecting the results of the clinical and immunological efficacy of Proteflazid® was carried out according to the following statistically significant indicators:

I. Trend data of cellular immunity indicators during the drug Proteflazid® administration in the treatment of diseases caused by HHV infections in pregnant women:

1. Relative percentage of T-lymphocytes (CD3+) in the blood of pregnant women, %.
2. Relative percentage of T-helper inductors (CD4+) in the blood of pregnant women, %.
3. Relative percentage of B-lymphocytes (CD19+) in the blood of pregnant women, %.

II. Gestation course in pregnant women with HHV infections during treatment with the drug Proteflazid®:

1. Incidence rate of delivery at term in women with HHV infections.
2. Incidence rate of threatened miscarriage in women with HHV infections.
3. Incidence rate of spontaneous miscarriages in women with HHV infections.

The clinical results on these indicators are presented in tab. 2-11.

Table 2. Relative T-lymphocytes (CD3+) percentage in pregnant women with HHV infections during the drug Proteflazid® administration

Author	Treatment group		Control group	
	Relative percentage of T-lymphocytes (CD3+) in pregnant with HHV infections who were administered Proteflazid®, %	Total number of patients	Relative percentage of T-lymphocytes (CD3+) in pregnant with HHV infections who were not administered Proteflazid®, %	Total number of patients
Azimova E.I., 2011	64,67±0,40	30	57,56±0,34	30
Nikolaeva S.V., 2007	64,78±0,409	20	57,47±0,215	20
Total	-	50	-	50

On the indicator “Relative percentage of T-lymphocyte (CD3+) in pregnant women with HHV infections during the Proteflazid® administration at the stage of the end of treatment,” 100 pregnant women with HHV infections were examined, 50 of whom were included to the treatment group and 50 – to the control group.

Table 3. Relative percentage of T-helper inductors (CD4+) in pregnant women with HHV infections during the drug Proteflazid® administration

Author	Treatment group		Control group	
	Relative percentage of T-helper inductors (CD4+) in pregnant with HHV infections who were administered Proteflazid®, %	Total number of patients	Relative percentage of T-helper inductors (CD4+) in pregnant with HHV infections who were not administered Proteflazid®, %	Total number of patients
Azimova E.I., 2011	39,7±0,23	30	35,4±0,24	30
Nikolaeva S.V., 2007	39,91±0,22	20	35,02±0,23	20
Total	-	50	-	50

On the indicator “Relative percentage of T-helper-inductors (CD4+) in pregnant women with HHV infections during the drug Proteflazid® administration”, 100 pregnant women with HHV infections were examined, 50 of whom were included to the treatment group and 50 – to the control group.

On the indicator “Relative percentage of B-lymphocyte (CD19+) in pregnant with HHV infections during the drug Proteflazid® administration”, 160 pregnant women with HHV infections were examined, 80 of whom were included to the treatment group and 80 – to the control group.

On the indicator “Incidence rate of delivery at term in women with HHV infections”, 94 pregnant women with HHV infections and possibility of delivery in term were examined, 59 of whom were included to the treatment group and 35 – to the control group.

On the indicator “Incidence rate of threatened miscarriage in women with HHV infections” during the administration of Proteflazid®, 94 pregnant women with HHV infections were examined, 59 of whom were included to the treatment group and 35 – to the control group.

Table 4. Relative percentage of B-lymphocytes (CD19+) in pregnant women with HHV infections during the Proteflazid® administration

Author	Treatment group		Control group	
	Relative percentage of B-lymphocytes (CD19+) in pregnant women who were administered Proteflazid®, %	Total number of patients	Relative percentage of B-lymphocytes (CD19+) in pregnant women who were not administered Proteflazid®, %	Total number of patients
Azimova E.I., 2011	13,48±0,23	30	11,98±0,04	30
Benyuk V.O., 2012	13,48±0,23	30	11,98±0,04	30
Nikolaeva S.V., 2007	13,49±0,219	20	11,47±0,158	20
Total	-	80	-	80

Table 5. Incidence rate of delivery at term in women with HHV infections

Author	Treatment group		Control group	
	Number of patients who were administered Proteflazid®, whose pregnancy ended with delivery at term	Total number of patients	Number of patients who were not administered Proteflazid®, whose pregnancy ended with delivery at term	Total number of patients
Nikolaeva S.V., 2007	31	40	9	20
Simrok V.V., 2003	19	19	13	15
Total	50	59	22	35

Table 6. Incidence rate of threatened miscarriage in women with HHV infections

Author	Treatment group		Control group	
	Number of patients who were administered Proteflazid® with threatened miscarriage	Total number of patients	Number of patients who were not administered Proteflazid® with threatened miscarriage	Total number of patients
Simrok V.V., 2003	2	19	3	15
Nikolaeva S.V., 2007	13	40	15	20
Total	15	59	16	35

On the indicator “Incidence rate of spontaneous abortions in women with HHV infections” during the administration of Proteflazid®, 100 pregnant women with HHV infections were examined, 60 of whom were included to the treatment group and 40 – to the control group.

Study outcomes and discussion

Evaluation of the clinical and immunological efficacy of Proteflazid® in the treatment of diseases caused by HHV infections in pregnant women.

Table 7. Incidence rate of spontaneous abortions in women with HHV infections

Author	Treatment group		Control group	
	Number of patients who were administered Proteflazid®, whose pregnancy ended with spontaneous abortion	Total number of patients	Number of patients who were not administered Proteflazid®, whose pregnancy ended with spontaneous abortion	Total number of patients
Bilyk N.M., 2005	0	20	3	20
Nikolaeva S.V., 2007	3	40	5	20
Total	3	60	8	40

A sample statistic of the main immunological indicators of pregnant women with HHV infections who were administered (treatment group) and who were not administered (control group) the drug Proteflazid® can be seen in tab. 8.

Table 8. Sample statistics of the main immunological indicators of pregnant women with HHV infections who were administered (treatment group) and who were not administered (control group) the drug Proteflazid®

Parameter		Mean	N	Standard deviation	Std. error of mean
Pair 1	Total number of CD3+ (T-lymphocytes) in pregnants with HHV infections who were administered Proteflazid®, %	64,7250	2	0,07778	0,05500
	Total number of CD3+ (T-lymphocytes) in pregnants with HHV infections who were not administered Proteflazid®, %	57,5150	2	0,06364	0,04500
Pair 2	Total number of CD4+ (helper cells) in pregnants with HHV infections who were administered Proteflazid®, %	39,8050	2	0,14849	0,10500
	Total number of CD4+ (helper cells) in pregnants with HHV infections who were not administered Proteflazid®, %	35,2100	2	0,26870	0,19000
Pair 3	Total number of CD19+ (B-lymphocytes) in pregnants with HHV infections who were not administered Proteflazid®, %	13,4833	3	0,00577	0,00333
	Total number of CD19+ (B-lymphocytes) in pregnants with HHV infections who were not administered Proteflazid®, %	11,8100	3	0,29445	0,17000

The significance test results of the main immunological parameters in pregnant women with HHV infections who were and were not administered Proteflazid®, using the Student's t-test, are summarized in tab. 9 and figs 1-3.

The conducted computation showed the positive effect of the drug Proteflazid® administration on immunological parameters in pregnant women with diseases caused by HVI against the total number of CD3+ (T-lymphocytes), CD4+ (helper cells) and CD19+ (B-lymphocytes), since the statistical significance of Student's t-test is less than 0.05. The total number of CD3+ (T-lymphocytes), CD4+ (helper cells) and CD19+ (B-lymphocytes) in pregnant women with HHV infections (treatment group) is within normal limits. The total number of CD3+ (T-lymphocytes) in pregnant women with HHV infections who were administered Proteflazid® (treatment group) as compared with pregnant women who were not administered this drug (control group) increased on average by 12.5%. The total number of CD4+ (helper cells) in pregnant women with HHV infections of the treatment group as compared with pregnant women of the control group increased on average by 13.1%. The total number of CD19+ (B-lymphocytes) in pregnant women with HVI of the treatment group as compared

with pregnant women of the control group increased on average by 14.2%. These changes indicate a positive effect of the drug Proteflazid® on the immunity of pregnant women with HHV infections.

Table 9. Significance test results of the main immunological parameters of pregnant women with HHV infections who were (treatment group) and were not (control group) administered Proteflazid®, using Student’s t-test

Parameter		Differences				t	Degrees of freedom	Two-tailed significance	
		Mean	Standard deviation	Std. error of mean	95 % confidence interval for a difference between means				
					Lower bound				Upper bound
Pair 1	Total number of CD3+ (T-lymphocytes) in pregnants with HHV infections who were administered Proteflazid® Total number of CD3+ (T-lymphocytes) in pregnants with HHV infections who were not administered Proteflazid®	7,21000	0,14142	0,10000	5,93938	8,48062	72,100	1	0,009
Pair 2	Total number of CD4+ (helper cells) in pregnants with HHV infections who were administered Proteflazid® Total number of CD4+ (helper cells) in pregnants with HHV infections who were not administered Proteflazid®	4,59500	0,41719	0,29500	0,84667	8,34333	15,576	1	0,041
Pair 3	Total number of CD19+ (B-lymphocytes) in pregnants with HHV infections who were administered Proteflazid® Total number of CD19+ (B-lymphocytes) in pregnants with HHV infections who were not administered Proteflazid®	1,67333	0,30022	0,17333	0,92754	2,41913	9,654	2	0,011

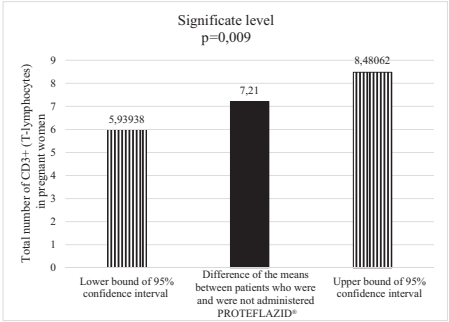


Figure 1. Significance test results of the indicator “Relative percentage of T-lymphocytes (CD3+) in pregnants with HHV infections who were (treatment group) and who were not (control group) administered Proteflazid®, using the Student’s t-test

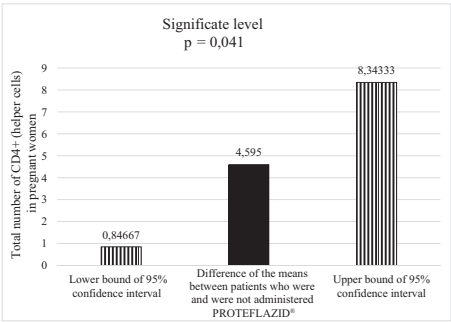


Figure 2. Significance test results of the indicator “Relative percentage of T-helper inducitors (CD4+) in pregnants with HHV infections who were (treatment group) and who were not (control group) administered Prote- flazid®, using the Student’s t-test

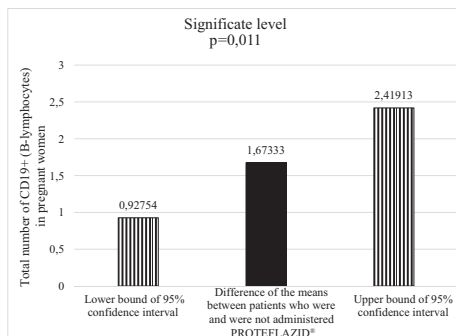


Figure 3. Significance test results of the indicator “Relative percentage of B-lymphocytes (CD19+) in pregnant women with HHV infections who were (treatment group) and who were not (control group) administered Proteflazid®”, using the Student’s t-test

Forest plot, which can be seen in fig. 4, shows the results of meta-analysis based on the odds ratio for the incidence rate of delivery at term in women with HHV infections in the treatment and control groups.

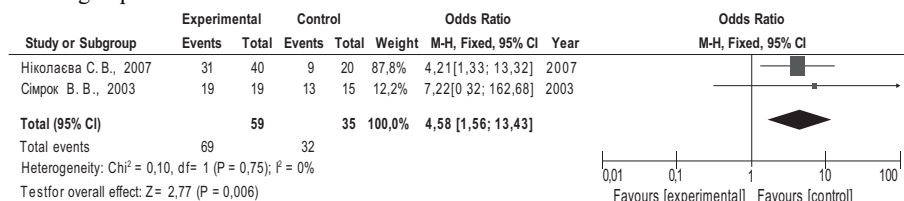


Figure 4. Results of a meta-analysis based on odds ratio of the incidence rate of delivery at term in women with HHV infections

The χ -square value of the criterion ($P=0.75$) and I^2 -test ($I^2=0\%$) indicate the homogeneity of these studies, therefore, a model with a fixed effect was chosen. The P-value of the Fisher criterion ($P = 0.006$) demonstrates the significance of selected effect.

The value of odds ratio ($OR=4.58$) indicates that probability of the incidence rate of delivery at term with a positive labour outcomes in women with in the treatment group is 4.58 times higher than in the control group.

The study of S.V. Nikolaeva (2007), which states that the Proteflazid® administration across pregnancy in patients with miscarriage associated with HHV infections, contributes to an incidence rate reduction in the HHV infections relapse by 70%, threatened miscarriage by 55%, placental dysfunction by 35%, preterm birth by 29.5% and total perinatal losses by 35% [12]. In the study of Simrok V.V. et al. (2003), it is noted that the gestation course in the treatment group when using the drug Proteflazid® is characterized by a significantly smaller number of obstetric complications, and ends, as a rule, by delivery at term with a birth of child in a satisfactory condition [17].

Forest plot of the meta-analysis results based on odds ratio of the incidence rate of threatened miscarriage in women with HHV infections after administration of Proteflazid® in the treatment and control groups can be seen in fig. 5.

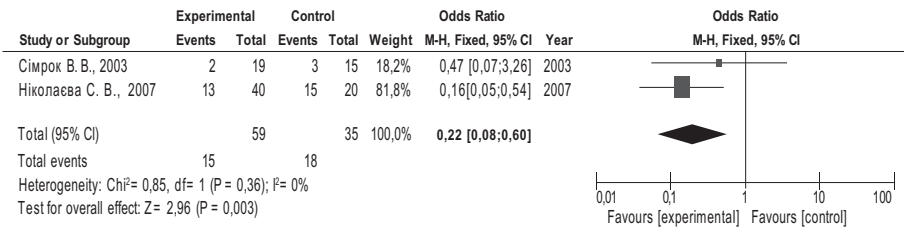


Figure 5. Results of the meta-analysis based on odds ratio of the incidence rate of threatened miscarriages in women with HHV infections

The χ -square value of the criterion ($P=0.36$) and I^2 -test ($I^2=0\%$) indicate the homogeneity of these studies, therefore, a model with a fixed effect was chosen. The P -value of the Fisher criterion ($P=0.003$) demonstrates the significance of selected effect.

The value of odds ratio ($OR=0.22$) indicates that probability of the incidence rate of threatened miscarriage in women with HHV infections after using the drug Proteflazid® in the treatment group is 4.55 times lower than in the control group.

The study of Nikolaeva S.V. (2007) has the greatest weight, which is pointed out that the inclusion of Proteflazid® in the comprehensive treatment for the preconception preparation of women with HHV infections and correction across pregnancy conduces to improving the immune system functions: an increase in the total number of Tand B-lymphocytes, an increase in the immunoregulatory index (CD4/CD8 cell ratio), a decrease in the CIC concentration, a decrease in IgG titres to HSV 1,2 types, the disappearance of IgM to HSV 1,2 types, a decrease in the viral replication intensity, and antigen load as well [12].

Forest plot of the meta-analysis results based on the odds ratio of the incidence rate of spontaneous abortions in women with HHV infections after using Proteflazid® in the treatment and control groups can be seen in fig. 6.

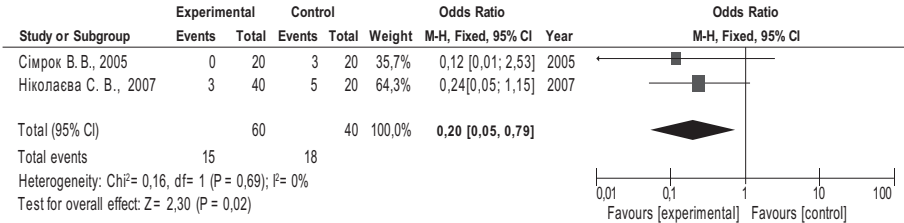


Figure 6. Results of meta-analysis based on odds ratios of the incidence rate of spontaneous abortions in women with HHV infections

The χ -square value of the criterion ($P=0.69$) and I^2 -test ($I^2=0\%$) indicate the homogeneity of these studies, therefore, a model with a fixed effect was chosen. The P -value of the Fisher criterion ($P=0.02$) demonstrates the significance of selected effect.

The value of odds ratio ($OR=0.20$) indicates that probability of the incidence rate of spontaneous abortions in pregnant women with HHV infections after using Proteflazid® in the treatment group is 5 times lower than in the control group.

According to this indicator, the most significant is the study of Nikolaeva S.V. (2007). In a study conducted by Bilyk N.M. (2005), it is noted that pregnant women who were administered Proteflazid®, as part of comprehensive therapy, the number of deliveries at term is twice as high

as compared with the control group and, accordingly, the incidence rate of missed miscarriages and spontaneous abortions – two times lower [5].

Sensitivity analysis

The sensitivity analysis was conducted in such a way as to assess the effect of each particular study on the integrated data, without taking into consideration case studies. The sensitivity analysis results showed that not a single case study significantly affected the integrated data, which indicates statistically significant results.

Funnel plot was used to assess the publication bias included in the study (figs 7-9). Virtually all ES values in figs 7-9 are within the funnel, which indicates unbiasedness.

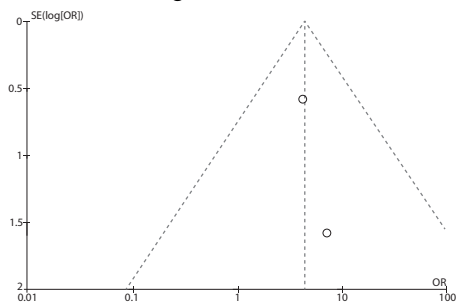


Figure 7. Funnel plot for the indicator “Incidence rate of delivery at term in women with HHV infections”

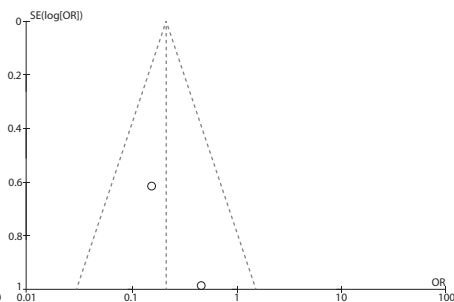


Figure 8. Funnel plot for the indicator “Incidence rate of threatened miscarriage in women with HHV infections”

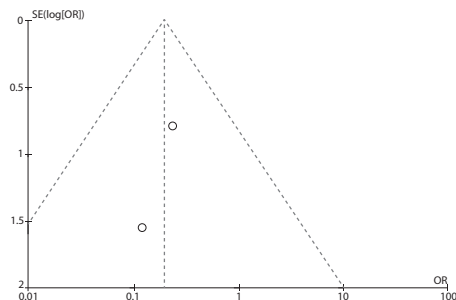


Figure 9. Funnel plot for the indicator “Incidence rate of spontaneous abortions in women with HHV infections”

Conclusions

Evaluation of the drug Proteflazid® efficacy using Student’s t-test proves: in pregnant women infected with HHV infections who were administered Proteflazid® (treatment group), as compared with pregnant women who were not administered this drug (control group), the relative percentage of T-lymphocytes (CD3+) increases on average by 12.5%, T-helpers (CD4+) – by 13.1%, B-lymphocytes (CD19+) – by 14.2%.

The meta-analysis conducted showed that the probability of the incidence rate of deliveries at term with a positive labour outcome in women with HHV infections during therapy with Proteflazid® in the treatment group is 4.58 times higher than in the control one; the

probability of the incidence rate of threatened miscarriage in women with HHV infections in the treatment group was 4.55 times lower than in the control group; the probability of the incidence rate of spontaneous abortions in the treatment group was 5 times lower than in the control one.

In the scientific publications, subject to analysis, there is no information concerning the toxic, mutagenic, teratogenic effects of Proteflazid®, which confirms the safety of its use in obstetric practice.

The meta-analysis and analysis based on Student's t-test confirm the efficacy of Proteflazid® in the treatment of diseases caused by HHV infections in pregnant women. Therapy with the drug Proteflazid® is etiologically and pathogenetically substantiated, contributes to the normalization of cellular immunity parameters, which, in turn, has a positive effect on the course and outcome of pregnancy.

References:

1. Венцовський В. Протефлазид® в лікуванні та профілактиці герпесвірусної інфекції у вагітних жінок: метааналіз клінічних результатів / В. Венцовський, О. Гриневич, В. Михайлов [et al.] // American Journal of Science and Technologies. – 2016. – №1 (21), January-June. – С. 983–1002.
2. Азімова Е. І., Султанов С. М., Нішанова Ф. П. До питання рецидиву генітального герпесу у вагітних з антенатальною загибеллю плода в анамнезі // Здоров'я жінки, 2011. – №2 (58). – С. 238–240.
3. Беньок В. О., Діндар О. А., Ліхолой П. О. Периконцепційна профілактика та лікування герпесвірусної інфекції у вагітних із метаболічним синдромом // Здоров'я жінки, 2012. – №9 (75). – С. 116–120.
4. Беньок В. А. Кувіта Ю. В., Гриневич О. Й. та ін. Протефлазид®: метааналіз результатів клінічних досліджень з оцінки ефективності та безпеки застосування у вагітних / Здоров'я жінки. – 2014. – №7 (93). – С. 166–176.
5. Білик Н. М. Діагностика та лікування ретрохоріальних та ретроплацентарних гематом у жінок з антифосфоліпідним синдромом та вірусною інфекцією / Педіатрія, акушерство та гінекологія – 2005. – №2. – С. 91–94.
6. Вдовиченко Ю. П., Баскаков П. М., Глазков І. О. Застосування Протефлазиду для профілактики акушерських та перинатальних ускладнень у жінок із герпетичною інфекцією/Репродуктивне здоров'я жінки. – 2003. – №1 (13). – С. 38–40.
7. Геник Н. І. Протефлазид® у комплексній терапії ускладнень вагітності у жінок з хламідійною інфекцією / Репродуктивне здоров'я жінки. – 2002. – №3 (12). – С. 13–16.
8. Знам'янська Т. К. TORCH-інфекції в акушерстві та неонатології / За редакцією д-ра мед. наук, проф. Т. К. Знам'янської. – К., 2008. – 200 с.
9. Імунологія / За ред. Кузнецової Л. В., Бабаджана В. Д., Харченко Н. В. – Вінниця. – ТОВ «Меркьюрі-Поділля». – 2013. – 565 с.
10. Камінський В. В. Протефлазид® у вагітних: системний огляд результатів післяреєстраційних спостережень за безпечністю та ефективністю застосування / В. В. Камінський, М. М. Шалько, О. Й. Гриневич // Здоров'я жінки. – 2014. – №6 (92). – С.160–164.
11. Нагорна В. Ф., Ніколаєва С. В. Механізм імунонаправленої дії Протефлазиду під час вагітності у пацієнток з невиношуванням вагітності герпесвірусної етіології // Здоров'я жінки, 2007. – 1 (29). – С. 111–114.

12. Ніколаєва С. В. Прегравідарна підготовка та ведення вагітності у пацієнток з невиношуванням вагітності на тлі герпесвірусної інфекції: Автореферат дис. канд. мед. наук: спец. Акушерство та гінекологія: 14.01.01 / С. В. Ніколаєва. – Одеса, 2007. – 25 стор.
13. Островська О. Н. Деякі аспекти застосування препарату Протефлазид® у комплексному лікуванні загрози переривання вагітності у жінок з хламідійною інфекцією // Репродуктивне здоров'я жінки. – 2 (11). – 2002. С. 9–11.
14. Островська О. М. Клініко-патогенетичні аспекти невиношування вагітності у жінок із хламідійною інфекцією та їх медикаментозна корекція: Автореферат дис. канд. мед. наук: спец. 14.01.01 – акушерство та гінекологія. / О. М. Островська. – Київ, 2005. – 22 стор.
15. Прокопюк В. Ю. Вплив мікоплазмової інфекції на систему мати-плацента-плід та шляхи корекції патологічних змін: Автореферат дис. канд. мед. наук: спец. Акушерство та гінекологія: 14.01.01 / В. Ю. Прокоп'юк. – Харків, 2009. – 20 стор.
16. Резніченко Н. А. Імуномодулятор у лікуванні перинатальних інфекцій // Питання експериментальної та клінічної медицини, 2013. Вип. 17, том 2. – С. 3–9.
17. Сімрок В. В., Гордієнко О. В. Ефективність Протефлазиду у профілактиці вірусних інфекцій у вагітних / Збірник наукових праць «Проблеми екологічної та медичної генетики та клінічної імунології». – Київ – Луганськ – Харків. – 2003. – С. 347–356.
18. Танько О. П., Потапова Л. В., Самойлова Д. В. Сучасні позиції імунотерапії при патології шийки матки у вагітних // Репродуктивне здоров'я жінки, 2006. – 3 (28). – С. 136–138.
19. Танько О. П., Скорбач Ю. І. Імуномодуюча терапія вульвовагінітів у вагітних // Репродуктивне здоров'я жінки, 2007. – №1 (30). – С. 90–92.

Посилання: Beniuk V., Kuznetsova L., Grynevych O., Broun T., Momotiuk L., Motuzka O., Yashchenko L. // *Proteflazid®: outcome analysis of clinical trials studying the impact of human herpesvirus infection on some cellular immunity indicators and gestation course in women* // *Pol. Med. J.* – 2019. – XLVI (274). – P. 157–164.

6.4. PREVENTION OF PERINATAL COMPLICATIONS IN PREGNANCY WITH CHRONIC PAPER VIRUS INFECTION

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Abstract

Social trends, the new style of the modern young woman, and the polygamy of sexual relations increase the frequency and likelihood of sexually transmitted infections including the human papilloma virus. In women who enter maternity with chronic papillomavirus infection, pregnancy is accompanied by an increased level of obstetrics and perinatal complications. The impossibility of antiviral drugs using due to their embryo- and fetotoxic effects requires the search of safe preventive measures against transmission of papilloma virus infection, timely and effective pre gravidarum and prenatal preparation.

The aim is to reduce the level of obstetric and perinatal complications in pregnant women with chronic papilloma virus infection by using of adequate pre-gravidarum and prenatal preparation.

Materials and methods. T- and B-lymphocyte, proinflammatory (IL-2, IL-6, TNF- α) and anti-inflammatory (IL-4, IL-10) cytokines levels and a follow-up analysis of pregnancy and childbirth were evaluated in 89 women with chronic papillomavirus infection

Results. Pregnancy course in women with chronic papillomavirus infection is accompanied by a higher incidence of perinatal complications such as placental dysfunction, polyhydramnios, preeclampsia, PROM, preterm birth, oligohydramnios, IUGR. Chronic papillomavirus infection causes a decrease in CD3+, CD 4+, and CD 19+, elevated level of CD 8+, activation of proinflammatory cytokines (IL-2, IL-6, TNF- α), decreased anti-inflammatory concentrations their analogues (IL-4, IL-10) during pregnancy, which is evidence of a pronounced secondary immunodeficiency condition. This, in turn, creates favorable conditions for the reactivation of viral infection, and in the future cause reproductive complications and losses.

Conclusions. The use of herbal drug PROTEFLAZID® which contain *Herba Deschampsia caespitosa* L. and *Herba Calamagrostis epigeios* L. for the pre-gravid and prenatal preparation in women, with chronic papillomavirus infection contributes to the restoration of the immune balance of pregnant women and certain decrease of perinatal complications.

Key words: chronic papillomavirus infection, PROTEFLAZID®, pregnancy, perinatal complications.

Introduction

The main reasons for the decrease in the protective function of the immune system of a pregnant woman's body may be active and/or passive smoking, immunodeficiency state, including HIV/AIDS, low social level, including lack of hygiene skills, low availability of quality medical care, long-term infection with certain types of human papillomavirus (HPV)

(70% of 16 and 18 types), early onset sexual activity (up to 16 years), frequent change of sexual partners, and the presence of other sexually transmitted infections, which increase the risk of HPV infection and impair the protective mechanisms for the cervical epithelium [2]. The studies of D. Lopez-Hernandez et al. (2016) [3] showed that in response to the papillomavirus invasion into a healthy organism, the immune system reacts and, in the absence of favourable conditions for the viral development, its self-elimination occurs in 80% of cases. With age (after 30 years) the probability of viral self-elimination properties are decrease, respectively increase the risk of developing precancer and cervical cancer. The virus begins to integrate into the chromosomal apparatus of the cell, which is a necessary condition for its replication. Clinically, this is manifest by cervical epithelial dysplasia, the severity of which in the absence of treatment is slow but steadily progressing. The final manifestation of virus aggression is the development of cervical cancer [4].

The tendencies of modern society indicate the postponement of motherhood for building a career, self-realizing in the professional sphere, obtaining social welfare, etc. [8]. Literature data point out that in most countries of the European Union, the mean age of the mother at her first childbirth is gradually increasing from 28.7 to 29.1 years from 2013 to 2017. According to the literature, in most European Union countries the average age of birth of a first child is gradually increasing from 28.7 to

29.1 years from 2013 to 2017. According to sociological surveys, postponement of maternity is also observed in Ukraine, thus in 2017 the average age of a mother at birth was 25 years old, while in 2010 it was 22 years old [9]. Such social tendencies, lifestyle of the modern young woman, in particular polygamy of sexual relations increase the frequency and probability transmission of infection with sexually transmitted pathogens, including HPV. In the end, there is a situation when a woman firstly becomes a carrier of HPV, which, in the absence of appropriate medical observation, provokes the development of a precancerous condition, and then she considers the realization of motherhood. Thus, there is a group of women entering motherhood who have chronic HPV carriers, who's a particular immunological condition during pregnancy may contribute to the activation of viral infection [1].

However, it should be noted that treatment with chemical antiviral drugs is strictly contraindicated during pregnancy due to their embryonic and fetotoxic effects. Therefore, if faced with pregnancy complications in this group of women, only symptomatic therapy it is possible to apply. Despite the viral nature of this condition, the inability of complete viral self-elimination and the high probability of their activation during pregnancy, there is a high probability of fetal contact and its infecting, so adequate preconceptional and prenatal preparation should be developed to address the issue of such patients [10].

The aim of the study

Reduce the level of obstetrics and perinatal complications in pregnant women infected with the human papillomavirus by applying adequate preconceptional and prenatal preparation.

Materials and methods

A follow-up analysis of pregnancy and childbirth was performed in 89 women. All women were followed up in the maternity clinic concerning CIN I-II, had chronic HPV carriage and had previously received treatment with antiviral drugs, endogenous interferon inducers or immunomodulators and, if necessary, surgical removal of atypically changed cervical epithelium. After the treatment, a control cytological examination confirmed the presence of unchanged epithelium.

In 36 patients (group I) to prevent the development of complications during pregnancy and childbirth, a special antiviral drug Proteflazid („SMC „Ecoparm”, LTD Ukraine) that contain a complex of herbal flavonoids was used in a special scheme. So, for the purpose of pre-conceptional preparation, proteflazid was used per oralis by 12-15 drops 2 times a day for 14 days, and for the purpose of prenatal preparation this drug was applied in the form of vaginal suppositories 1 time a day, since 36-37 weeks for 14 days. 28 patients (group II) were admitted to the maternity clinic after confirmation of pregnancy; consequently, they did not receive periconceptional preparation. 25 pregnant women, without chronic HPV carriage and with unchanged cervical epithelium, formed a control group.

The examination of all pregnant women was performed in the accordance with the current recommendations, regulated by the Order of the Ministry of Health of Ukraine “On Organization of Outpatient Obstetrics-Gynecological Care in Ukraine” dated 15/07/2011 No. 417.

Laboratory and immunological tests were performed before the onset of pregnancy and directly before childbirth.

The immune status was evaluated by the determination of the number of Tand B-lymphocytes, using the indirect variant of the immunofluorescence method with monoclonal antibodies against CD3+, CD4+, CD8+, CD19+-lymphocytes (LabSystems Multiskan MCC/340) using the Seiler H 6000 Series fluorescent microscope. In addition, the immunoregulatory index (CD4+/CD8+) and the concentration of circulating immune complexes (CIC) were determined using a spectrophotometer. The obtained results were evaluated in combination with the data of paraclinical examinations and clinical manifestation of the disease.

The pro-inflammatory (IL-2, IL-6, TNF-a) and anti-inflammatory (IL-4, IL-10) factor concentration in the blood serum was determined using the enzyme-linked immunosorbent analyser (ELISA): STAT FAX 303 PLUS (USA) and assay kits and test systems to quantify them.

The statistical processing of the results was performed using the statistical analysis programs Microsoft Excel 7.0 and Statistica for Windows 6.0. The results are given as mean values and their standard errors ($M \pm m$). Student's t-test was used to assess the significance of changes between the comparison groups.

Table 1. The state of cellular and humoral components of the immune system in pregnant women with chronic papillomavirus infection under the influence of treatment ($M \pm m$)

Indicators	I group, n=36		II group (n=28)	Control group, (n=25)
	Before treatment	After treatment		
CD3+,%	51,2±2,6	61,3±3,2 **	53,2±2,2 *, **	71,2±3,9
CD4+,%	32,1±2,4	39,3±2,9 **	31,1±2,1 *, **	41,4±2,9
CD8+,%	37,4±2,5	31,8±2,2 **	38,3±2,3 *, **	27,4±2,5
CD4+ /CD8+	0,85±0,1	1,24±0,2	0,81±0,2*	1,51±0,2
CD19+,%	19,1±1,9	26,3±1,9	19,4±2,1*	27,3±2,0
CIC, /ODU	49,1±4,0	46,2±3,9	48,2±4,1	41,4±3,1

* indicators significantly differ compared with the control ($p=0.05$)

** indicators significantly differ in the treatment results ($p=0.05$).

Results and discussion

The average age of the examined women was 31.2 ± 2.1 years. In 50 (56%) women it was the first pregnancy, while in 39 (44%) it was the second one. The previous pregnancies ended in childbirth in 28 (31.5%) patients, in 8 (9%) of them through the caesarean section, in 7 (8%) – spontaneous abortions and in 4 (4.5%) – artificial abortions.

The results of immunological studies in the examined groups of pregnant women are presented in table 1.

In the women with chronic HPV carriers, before pregnancy, there was a significant decrease in numbers of CD3+ (T-lymphocytes) to $51.2 \pm 2.6\%$ ($p \leq 0.05$), CD4+ (T-helpers) to $32.1 \pm 2.4\%$ ($p \leq 0.05$), CD8+ (T-suppressors) to $37.4 \pm 2.5\%$ ($p \leq 0.05$) and CD19+ (B-lymphocytes) to $19.1 \pm 1.9\%$ ($p \leq 0.05$) compared to similar in controls.

The study showed that the pregnant women who did not receive preconceptional preparation during pregnancy also had a significant decrease in the total number of CD3+ (T-lymphocytes), which was $53.2 \pm 2.2\%$ versus $71.2 \pm 3.9\%$ ($p \leq 0.05$) in controls and in patients of group I who received proposed treatment $61.3 \pm 3.2\%$ ($p \leq 0.05$).

Similar changes occurred when evaluating the number of CD4+ (T-helpers) during pregnancy, its level in the pregnant women who did not receive preconceptional preparation was reduced to $31.1 \pm 2.1\%$ versus $39.3 \pm 2.9\%$ ($p \leq 0.05$) in patients in group I and in control $41.4 \pm 2.9\%$ ($p \leq 0.01$).

The CD8+ level (T-suppressors) was increased in women who didn't receive preconceptional preparation before and during pregnancy. It should be noted that this indicator was significantly higher in the group of women who did not receive preconceptional preparation $38.3 \pm 2.3\%$ ($p < 0.01$) compared with the control group $27.4 \pm 2.5\%$ and the group of women who received it $31.8 \pm 2.2\%$ ($p < 0.05$), moreover, in the group I and in the control this indicator were not significantly differ ($p > 0.05$).

Imbalance in the immune response of lymphocytes of pregnant group II was accompanied by a decrease in the immunoregulatory index CD4+/CD8+ compared with the control group. In pregnant of group I. who received preconceptional preparation, significant changes of indicators of immune response were not found. This indicates about compensative immune response for circulation of papilloma virus after using of preconceptional preparation.

Significant reduction of CD19+ (B-lymphocytes) number to $19.4 \pm 2.1\%$ occurred only in the patients who did not receive the preconceptional preparation, compared with the control ($27.3 \pm 2.0\%$) ($p < 0.01$) and the group of women who receive such treatment – $26.3 \pm 1.9\%$ ($p < 0.05$).

The CIC level in both groups has tendency to increase compared to the control group, but did not differ significantly between all groups, indicating a low activity of the B-system of the humoral immune system. At the same time, it should be emphasized that this indicator also has tendency to decrease in the group of pregnant women who received the preconceptional preparation compared with the group of pregnant women who did not receive this treatment.

Thus, the results of the study showed the presence of immune imbalance which characterized by a reducing the total number of T-lymphocytes (CD3+), T-helper (CD4+) and B-lymphocytes (CD19+) along with an increased T-suppressor (CD8+) number. It should be noted that the misbalanced immune response balance were observed in the patients of both groups, however, their severity was higher in those women who did not receive proteflazid during the course of the preconceptional preparation. At the same time, it should be noted

that pregnancy is a specific immunological condition, in which certain components of the immune protection are significantly reduced thus the pregnancy is carried out. On the other hand, such changes in the immunological status may contribute to the reactivation of the viral infection and cause the reproductive losses.

We also investigated the interleukin pool, which is considered to be a regulator of the functional activity of the body's immune system. As can be seen from table 2, in patients, who received in the complex of preconceptional preparation were only a significant decrease in the concentration of IL-2 to 2.0 ± 0.2 pg/ml ($p < 0.001$) compared with untreated women 3.0 ± 0.3 pg/ml. However, its level was almost 2 times higher than in the control group (1.1 ± 0.1 pg/ml) ($p \leq 0.05$). The concentration levels of other proinflammatory (IL-6, TNF- α), as well as anti-inflammatory (IL-4, IL-10) cytokines in the treated group was not significantly different from similar indicators of the control, which indicates high efficiency of proposed treatment and its prolong duration of positive effects on the regulation of nonspecific immune response in pregnant women.

Table 2. Pro- and anti-inflammatory interleukin concentration levels in the examined pregnant women (M $\pm$ m)

Indicators	I group, n=36		II group (n=28)	Control group (n=25)
	Before treatment	After treatment		
IL-2, pg/mL	3,1 $\pm$ 0,3	2,0 $\pm$ 0,2*	3,0 $\pm$ 0,3*,**	1,1 $\pm$ 0,1
IL-6, pg/mL	9,3 $\pm$ 0,9	6,8 $\pm$ 0,6	9,8 $\pm$ 0,9*,**	5,3 $\pm$ 0,5
TNF- α , pg/mL	11,1 $\pm$ 1,0	7,3 $\pm$ 0,7	10,6 $\pm$ 0,9*,**	6,7 $\pm$ 0,6
IL-4, pg/mL	3,1 $\pm$ 0,4	6,3 $\pm$ 0,6	3,2 $\pm$ 0,3*,**	5,2 $\pm$ 0,5
IL-10, pg/mL	2,5 $\pm$ 0,4	5,2 $\pm$ 0,4	2,4 $\pm$ 0,5*,**	4,4 $\pm$ 0,4

* indicators significantly differ compared with the control ($p=0.05$)

** indicators significantly differ in the treatment results ($p=0.05$).

The study found that in pregnant women who had not received HPV antiviral treatment, the concentration levels of proinflammatory interleukins were significantly higher than in those who received it. Thus, IL-2 and IL-6 levels were 3.0 ± 0.3 pg/ml and 9.8 ± 0.9 pg/ml respectively, and were nearly 1.5 ($p \leq 0.05$) and 1,4 times ($p \leq 0.05$) respectively higher compared to pregnant women who received preconceptional preparation and 2 times higher ($p < 0.001$) compared to controls. TNF- α level was 10.6 ± 0.9 pg/ml and was also significantly higher compared to the control group ($p < 0.001$) and the group of women who received preconceptional preparation.

In HPV-infected pregnant women, who did not receive preconceptional preparation, the concentration levels of antiinflammatory cytokines remained significantly low. Thus, the levels of IL-4 and IL-10 were 3.2 ± 0.3 pg/ml and 2.4 ± 0.5 pg/ml, respectively, and 1.6 and 1.8 times lower than in the control group ($p < 0.01$) and 2 times lower than in patients who received preconceptional preparation ($p < 0.001$).

Therefore, in pregnant women who were chronic carriers of HPV, activation of proinflammatory cytokines was observed along with a decrease in the concentration of anti-inflammatory analogues compared to the control group. The results of the study also showed that the lack of preconceptional preparation in patients whose pregnancy occurs on the background of chronic HPV infection leads to the formation of immunological imbalances, which can play a leading role in the reactivation of

viral infection, which in turn can cause development of some perinatal complications. However, most researchers note that the realization of viral infection is due to infection with syncytiotrophoblast and a direct effect on the placenta [5]. In addition, fetal infection is most likely to occur ascending through the placenta and, in particular, during conception through sperm [1]. However, the results of studies on the possibility of infection of the fetus during the delivery are quite controversial, since the frequency of vertical transmission varies in a wide range from 4 to 87% [6,7].

It is also should be noted that certain features of HPV infection, in which the human immune defense is inadequate, since the virus replicates only in the epithelium, does not enter the blood and does not cause inflammation, and produces proteins that suppress only local immunity. Therefore, about half of infected women do not produce sufficient levels of antibodies against HPV, and if antibody levels are determined, they are extremely low and, along with immune imbalance, do not guarantee protection against HPV reactivation. Another feature is that HPV is highly associated with the cylindrical epithelium of the cervical canal, where major pathological changes occur, and the cervix and cervical canal are active participants of the delivery process, and it is that part of the uterus where major structural changes occur. The direct contact of the fetus with infected tissues during the delivery justifies the need to use safe for use in pregnancy antiviral drugs (proteflazid – suppositories) in the complex of prenatal preparation in such women. It should be emphasized that traditional therapy of HPV with immunostimulants and antiviral drugs is strictly contraindicated during pregnancy due to their embryotoxic and teratogenic effect on the fetus [1].

Our analysis of pregnancy course in HPV-infected women showed that all pregnant women who were carriers of HPVs had an increased risk of developing a number of such perinatal complications as placental dysfunction (49.4%), polyhydramnios (19.1%), preeclampsia (12.4%), premature rupture of the membrane (10.1%), preterm birth (9.0%), oligohydramnios (9.0%), fetal growth retardation syndrome (7.9%), and miscarriage (3.4%).

It should be noted that the incidence of perinatal complications in the group of women who received proteflazid in the complex of preconceptional and prenatal preparation was significantly lower (fig. 1).

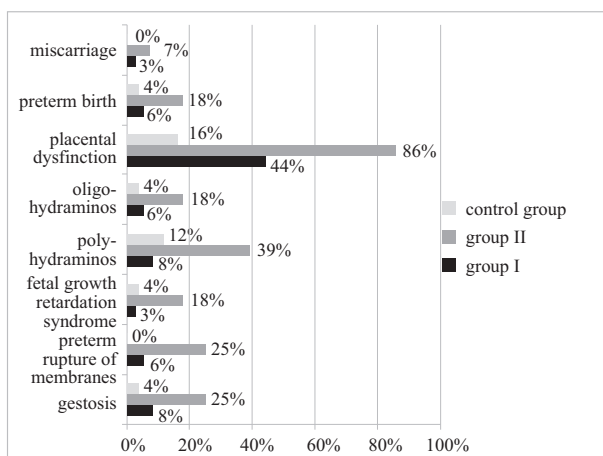


Figure 1. Frequency of perinatal complication in women with HPV

It was also found that in patients who did not receive pregravid preparation (group II), placental dysfunction developed 2 times more often (86% vs. 44%, $p<0.001$), polyhydramnios – almost 5 times more often (39% vs. 8%, $p<0.01$), premature rupture of membranes – more than 4 times more frequent (25% vs. 6%, $p<0.05$) and fetal growth retardation syndrome – 6 times more frequent (18% vs. 3%, $p<0.05$) than in patients of group I who received it. In addition, late gestosis (3 times), preterm birth (3 times) and oligohydramnios (3 times) were more often diagnosed in group II than in pregnant group I after appropriate proteflazid treatment.

In all, pregnancy completed by term delivery in 32 (89%) patients of group I, in 21 (75%) – of group II and in 24 (96%) – of control. The childbirth was complicated by the hypotonic uterine activity in 3 (8%) women of group I, in 5 (18%) – of group II and 1 (4%) – of control group. Fetal distress occurred in 2 (5.5%) patients of group I, in 4 (14%) – of group II.

Thus, the inclusion in the complex of prenatal preparation of proteflazid in women of group I made it possible to significantly reduce the development as perinatal as well as obstetrics complications in comparison with pregnant women who did not receive such therapy (group II).

Summarizing the results of the study, we can conclude that the inclusion in the programs of preconceptional and prenatal preparation the drug proteflazid which contain complex of plants' bioflavonoids, even on the background of the immune imbalance that is prevalence during pregnancy, inhibits the activation of papillomavirus infection, promotes the elimination of the viruses and prevent the development of such perinatal complications as placental dysfunction, polyhydramnios, which, in turn, is the cause of intrauterine infection of the fetus and its growth retardation.

According to the results of this study, a significant positive effect of proteflazid (drops and suppositories) on the course of pregnancy in the women infected with HPV was found. It can be argued that the positive clinical result can be explained by the specific direct antiviral action of the drug on the inhibition of virus-specific enzymes polymerases [11]. It was also established that proteflazid has the pronounced immune modulating properties by stimulating the production of pro-inflammatory and anti-inflammatory cytokines, which increase the immune resistance to viral and bacterial infections. In addition, considering the fact that proteflazid contains a large amount of bioflavonoids, has powerful antioxidant properties, inhibits freeradical oxidation processes and promotes the recovery of the body after infection. So, the use of specific antiviral therapy with proteflazid is not only effective but also safe for the fetus.

Conclusions

The HPV-infected pregnant women who did not receive in the complex of preconceptional and prenatal preparation had a significantly higher incidence of perinatal complications such as placental dysfunction (86%), polyhydramnios (39%), preeclampsia (25%), premature rupture of membranes (25%), preterm birth (18%), oligohydramnios (18%), and fetal growth retardation syndrome (18%).

In HIV-infected pregnant women were revealed a pronounced immune imbalance, which is manifested by a decrease number of CD3+, CD4+, and CD19+ along with elevated CD8+ numbers, and activation of proinflammatory cytokines (IL-2, IL-6, TNF- α) along with decreased concentration their anti-inflammatory analogues (IL-4, IL-10) and creates favorable conditions for reactivation of viral infection, development of perinatal complications and reproductive losses.

Carrying out in the complex of preconceptional and prenatal preparation of HPV-infected pregnant proteflazid (drops and suppositories) is accompanied by a significant decrease in the frequency of development of perinatal and obstetrics complications due to its specific antiviral effect on HPV, antioxidant properties and restoration of the immune balance in the pregnant woman.

References:

1. Bober L., Guzowski G., Moczułska H., Sieroszewski P. Influence of human Papilloma Virus (hPV) infection on early pregnancy. *Ginekol Pol.* 2019;90(2):72–75.
2. Jalilvand S., Shoja Z., Nourijelyani K., Tohidi H. R., Hamkar R. Meta-analysis of type-specific human papillomavirus prevalence in Iranian women with normal cytology, precancerous cervical lesions and invasive cervical cancer: implications for screening and vaccination. *J Med Virol.* 2015; 87(2):287–295.
3. López-Hernández D., Beltrán-Lagunes L., Brito-Aranda L., López-Hernández Mde L. Human papillomavirus infection and its correlates with clinically relevant gynecological and obstetric conditions: A cross-sectional study]. *Med Clin (Barc).* 2016 Aug 5; 147(3):101–8.
4. Mercado Gutiérrez M. R., Arean Cuns C., Gómez Dorronsoro M. L., Paniello Alastruey I., Mallor Giménez F., Lozano Escario M. D., Santamaría Martínez M. / Influence of Age in the Prevalence of High-Risk Human Papilloma Virus in Women with Pre-Neoplastic Cervical Lesions in Navarra, Spain]. *Rev Esp Salud Publica.* 2017 Feb 9; 91.
5. Mosbah A., Barakat R., Nabel Y., Barakat G. High-risk and low-risk human papilloma virus in association to spontaneous preterm labor: a case-control study in a tertiary center, Egypt. *J Matern Fetal Neonatal Med.* 2018 Mar; 31(6):720–725.
6. Nimrodi M., Kleitman V., Wainstock T., Gemer O., Meirovitz M., Maymon E., Benshalom-Tirosh N., Erez O. The association between cervical inflammation and histologic evidence of HPV in PAP smears and adverse pregnancy outcome in low risk population. *Eur J Obstet Gynecol Reprod Biol.* 2018 Jun; 225:160–165.
7. Rahimkhani M., Mordadi A., Gilanpour M. Detection of urinary Chlamydia trachomatis, Mycoplasma genitalium and human papilloma virus in the first trimester of pregnancy by PCR method. *Ann Clin Microbiol Antimicrob.* 2018 Jun 4; 17(1):25.
8. Salcedo M. M., Damin A. P., Agnes G., Pessini S. A., El Beitune P., Alexandre C. O. Prevalence of human papillomavirus infection in pregnant versus non-pregnant women in Brazil. *Arch Gynecol Obstet.* 2015; 292(6):1273–1278.
9. Shi N., Lu Q., Zhang J., Li L., Zhang J., Zhang F., Dong Y., Zhang X., Zhang Z., Gao W. Analysis of risk factors for persistent infection of asymptomatic women with high-risk human papilloma virus. *Hum Vaccin Immunother.* 2017 Jun 3; 13(6):1–7.
10. Ford J. H., Li M., Scheil W., Roder D. Human papillomavirus infection and intrauterine growth restriction: a data-linkage study. *J Matern Fetal Neonatal Med.* 2019 Jan; 32(2):279–285.
11. <https://compendium.com.ua/info/166711/proteflazid-sup-sup-kapli/>

Посилання: Heryak S., Petrenko N., Dobrianska V. Prevention of perinatal complications in pregnant women with chronic papillomavirus infection // *Polski Merkuriusz Lekarski.* – 2020. – №XLVIII (285). – P. 157–161.

6.5. MANAGEMENT OF PREGNANCY IN THE ASSOCIATION OF HIV AND HERPES VIRUS INFECTION

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Abstract

The recommended complex for management of HIV-infected pregnant women with herpes infection includes conducting the necessary studies, 2-stage prediction of the risk of obstetric and perinatal complications, and differential pathogenetically grounded treatment. There was proved the high effectiveness of the recommended complex. After the recommended treatment, there was observed the improvement of the indices of HIV course, reduction of toxicity of the body, improvement of bowel microecology and vaginal microbial landscape. The frequency of complications of pregnancy is reduced by 23.3%; placental insufficiency, preeclampsia, and fetal distress reduced by almost two times. The condition of children at birth significantly improves: 60.0% of children were born in satisfactory condition; the frequency of jaundice, hemorrhagic syndrome, and gastrointestinal syndrome is lower by 1.5–2 times; the frequency of life-threatening syndromes (neurological syndrome and SDRs) reduces by two times. The incidence of intrauterine infection with herpes viruses reduces by three times due to the use of specific immunoglobulins.

Key words: HIV, herpes virus infection, pregnancy, newborn, treatment, immunoglobulins.

Introduction

Herpes is one of the most common sexually transmitted infections, and it is included in a special study program conducted by the WHO [1]. Among HIV-positive individuals, the prevalence of herpes virus infection is significantly higher [2], which has increased the significance of this infection during the HIV epidemic in Ukraine. Against this background, herpes virus infection (HVI) is opportunistic and AIDS-indicative, and it is often considered the common cause of damage to the central nervous system and internal organs. Herpesviruses are particularly dangerous due to their clinical significance during pregnancy. Herpetic and cytomegalovirus infections belong to the TORCH group of diseases, which cause intrauterine pathologies [3, 4].

Treatment of herpes virus infection, which persists lifelong in the host's body, remains ambiguous and low effective. Modern medicine does not have treatment methods that would ensure the elimination of herpesviruses from the human body. Therefore, the goal of treatment is to suppress viral replication during exacerbations, to form an adequate immune response, and to maintain it over time in order to block virus reactivation in persistence sites [1].

The main approaches to HVI treatment include antiviral therapy with analogs of natural nucleosides (acyclovir, valacyclovir, famciclovir, etc.) and a comprehensive approach that combines immunotherapy (both specific and nonspecific) in combination with antiviral treatment [4, 7].

The choice of therapy during pregnancy is of particular significance, due to, on the one hand, the significant danger that herpes virus infection (HVI) poses to the fetus, the high risk of intra-uterine infection, and, on the other hand, the toxic effects and the unproven absence of teratogenic and embryotoxic effects of antiviral medicinal products. The usage of acyclovir-group medications in pregnant women is limited and allowed only under specific indications: primary HVI or recurrent HVI during the replication stage with severe clinical symptoms [5].

An even more complex and low studied issue is the treatment of HVI in pregnant women with HIV, due to immunodeficiency and the mutually reinforcing nature of the infections, HVI ability to activate HIV and increase viral load. In the presence of HIV, HVI is characterized by a severe clinical course, a pronounced tendency to recurrence, a higher frequency of acyclovir resistance, and abortive forms [2].

According to the literature and results of own studies, HVI in HIV-infected women increases the risk of obstetric and perinatal complications as well as the risk of vertical transmission of HIV from mother to child [1, 6]. In immunosuppressed patients, therapy only during exacerbation period does not significantly affect the course of HVI or reduce the indicated risks.

Study objective:

Evaluating the effectiveness of a management complex for pregnant women at the association of HIV and herpes virus infection.

Study materials and methods

The conducted comprehensive study made it possible to identify the main links in the pathogenesis of obstetric and perinatal complications in HIV-infected pregnant women with HVI, as shown in Figure 1. This allowed us to create a theoretical basis for developing a pathogenetically targeted therapeutic and preventive care management complex for such patients.

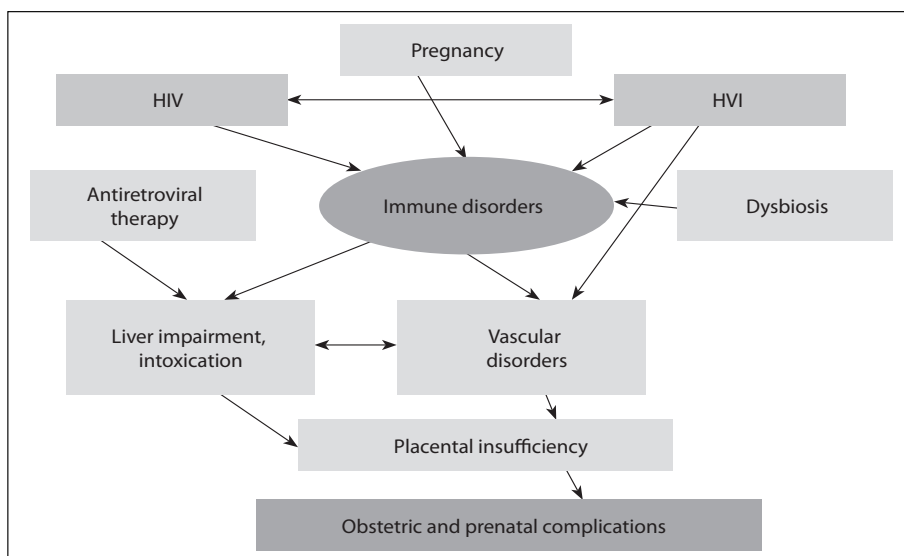


Figure. 1. Key links in the pathogenesis of obstetric and perinatal complications in HIV infection

Complex therapy for HIV-infected pregnant women with HVI should aim to reduce viral activity, inhibit viral replication and accelerate viral elimination; normalize factors of specific and nonspecific immunity; detoxify the body; improve vascular regulation and eliminate endothelial dysfunction; and normalize the microbiota.

The treatment scheme is presented in Figure 2.

Basic Complex	In case of high risk	Severe clinical manifestations
- PROTEFLAZID® (drops), which has antiviral and immunomodulatory effects – 4 weeks; - probiotic Lactostar Plus to eliminate systemic dysbiosis and restore local mucosal immunity — 1 capsule a day for 2 weeks; - Glutargin, considering its detoxifying and endothelium-protective properties – 1 tablet every 2 days for 2 weeks.	- Specific immunoglobulin against HSV-2 or CMV – 1 ampoule intramuscularly once every 3 days, 5 injections (in case of mixed infection – treatment with both immunoglobulins); - PROTEFLAZID® (drops) according to the 3-month scheme; in case of clinical manifestations of genital herpes – PROTEFLAZID® (suppositories) for 7–10 days; - Lactostar Plus – 1 capsule every 2 days for 2 weeks; - Glutargin – 1 tablet every 3 days for 3 weeks.	- intravenous immunoglobulin Bioven (high concentrations of specific IgG) – 8 mol (0.4 g)/kg daily for 2–3 days; - Viferon 500,000 IU, 1 suppository twice daily for 10 days; - PROTEFLAZID® (drops); in case of clinical manifestations of genital herpes – PROTEFLAZID® (suppositories) for 7–10 days; - Lactostar Plus; - Glutargin.

Figure 2. Treatment scheme for HIV-infected pregnant women with HVI

Considering the particular danger of HVI in HIV-infected pregnant women, we recommend including two therapeutic courses in the basic treatment complex for such patients: PROTEFLAZID® (drops) – exhibits antiviral and immunomodulatory effects: should be administered according to the standard dosing scheme for adults (treatment course duration: 4 weeks); Probiotic Lactostar Plus – to eliminate systemic dysbiosis and restore local mucosal immunity: 1 capsule once a day during 2 weeks; Glutargin – considering its detoxifying and endothelium-protective properties: 1 tablet (250 mg) twice a day during 2 weeks.

For women identified according to the results of the first stage of therapeutic prediction as having a high risk of perinatal and obstetric complications, an enhanced therapeutic and preventive care complex is proposed starting from the 14th week of pregnancy:

1. Human specific immunoglobulin against herpes simplex virus type 2 (HSV-2) or anti-cytomegalovirus immunoglobulin. If both herpes and cytomegalovirus infections (CMV) are present, both immunoglobulins are used. The specific human immunoglobulin is administered as follows: 1.5 ml of the medicinal product (the contents of 1 ampoule) administered intramuscularly once every 3 days, for a total of 5 injections over three courses. In cases of combined HSV and CMV infection, treatment with one immunoglobulin was administered according to the same scheme (1.5 ml intramuscularly once every 3 days, total 5 injections), and after a 2-day break – treatment with another immunoglobulin.

2. PROTEFLAZID® (drops): (7 drops twice daily for 3 days, 10 drops twice daily for the next 3 days, 12–15 drops twice daily for 3 months without interruption). In cases of pronounced local clinical manifestations of genital herpes (GH), additionally: PROTEFLAZID® (suppositories): 1 suppository once daily for 7–10 days or longer until symptoms resolve.

3. Lactostar Plus – 1 capsule twice daily (in the morning and in the evening) for 2 weeks.

4. Glutargin – 1 tablet (250 mg) three times a day for 3 weeks.

In cases of severe clinical manifestations of HVI, instead of specific immunoglobulin, intravenous normal human immunoglobulin Bioven is used, due to its high concentrations of specific IgG, at a dose of 8 ml (0.4 g/kg daily for 2–3 days).

Additionally, Viferon 500,000 IU is prescribed rectally: one suppository every 12 hours (twice daily) for 10 days, followed by one suppository every 12 hours (twice daily) twice a week – for 10 days.

At 30–31 weeks of pregnancy, the repeated therapeutic course is administered for all pregnant women with HIV and HVI, and in cases of high risk of perinatal and obstetric complications defined based on the results of the second stage of therapeutic prediction, an enhanced therapeutic and preventive care complex is applied.

To assess the effectiveness of the recommended therapeutic and preventive care complex, 60 HIV-infected pregnant women with HVI were selected, in whom a high risk of obstetric and perinatal complications was identified based on the developed prediction algorithm.

Using the method of randomization, 60 women were divided into two groups: the main group included 30 pregnant women receiving the recommended preventive care complex; comparison group included 30 pregnant women managed according to the protocols of the Ministry of Health of Ukraine

Results and Discussion

In the course of the recommended treatment, which included medicinal products with anti-herpesvirus and immunomodulatory effects, a trend toward improvement in HIV progression was observed: the proportion of patients with CD4⁺ count less than 350 cells/ μ L decreased twice (from 26.7% to 13.7%), and by 13.3% for those with CD4⁺ count less than 40%, which was not observed in the comparison group. The proportion of women with a viral load (VL) below 10,000 copies/mL increased from 36.7% to 60% ($p < 0.05$), which was significantly higher than in the comparison group – 26.7% ($p < 0.05$).

According to the results of biochemical blood analysis, a statistically significant decrease was found in bilirubin levels and cytotoxic markers: AST from 51.2 ± 3.4 to 35.3 ± 4.5 IU/l ($p < 0.05$), ALT from 39.7 ± 3.5 to 30.3 ± 2.5 IU/l ($p < 0.05$), which, along with a reduction in creatinine and urea levels, indicates a detoxifying effect of the recommended comprehensive treatment, due to improved liver function and normalization of intestinal biocenosis.

Following treatment involving probiotics, women in the main group showed improved intestinal microecology, as bacterial culture test showed the reduction of pathogenic microflora. According to laboratory and clinical indicators, the proportion of women in the main group with varying degrees of intestinal dysbiosis decreased from 66.7% to 46.7%. The vaginal microbial landscape also improved. Lactobacilli, whose presence is critical for diagnosing vaginal biocenosis disturbances, were detected in 13.3% and 16.7% of women before treatment, and in 46.7% ($p < 0.05$) and 20.0% after treatment, respectively. Consequently, the frequency of significant levels of pathogenic and conditionally pathogenic flora (Staphylococcus, Gardnerella, Chlamydia, Ureaplasma, Mycoplasma, and Candida fungi) also decreased as indicated by bacterial culture tests.

Improvement in women's general condition, HIV progression, vascular regulation, and overall microbiota contributed to better conditions for gestational development. Pregnancy complications were observed in 50.0% of women in the main group compared to 73.3% in the comparison group. The incidence of placental insufficiency decreased almost twice – from 46.7% to 26.7%, as well as preeclampsia incidence (Figure 3), and consequently, fetal distress decreased from 36.7% to 20.0%. There were no cases of maternal mortality; one case of perinatal mortality was identified in the comparison group (30.3%).

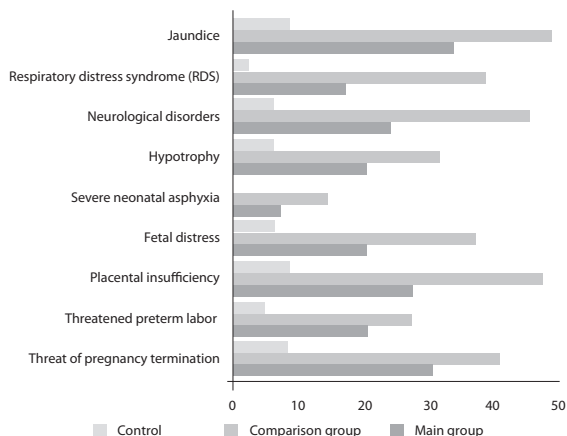


Figure 3. Frequency of obstetric and perinatal complications depending on the administered therapy

30 live babies were born in women of the main group, and 29 of the comparison group. At birth, the condition of the babies in the main group was significantly better: 60% were born in satisfactory condition (without asphyxia) compared to 37.9% ($p < 0.05$) babies in the comparison group. The proportion of babies with severe asphyxia was two times lower. The incidence of jaundice, hemorrhagic syndrome, and gastrointestinal syndrome was 1.5–2 times lower. It is important to note a statistically relevant reduction in the incidence of such life-threatening syndromes that may impair the child's further development as the neurological syndrome (down to 23.3% vs. 44.8%, $p < 0.05$) and craniosynostosis syndrome (decreased to 16.7% vs. 37.9%, respectively, $p < 0.05$).

In 3 babies (10.3%) from the comparison group, intrauterine pneumonia was diagnosed by the end of the first day of life.

PCR testing revealed the presence of HVI in 11 babies (37.9%) in the comparison group, and only in 4 babies (13.3%) – in the main group, which is attributed to the use of specific immunoglobulins.

Thanks to the complete antiretroviral therapy (ART) administered to all women, no cases of vertical HIV transmission from mother to child were reported.

Conclusion

The recommended complex for management of HIV-infected pregnant women with herpes virus infection involves conducting necessary diagnostic evaluations, the results of which are considered during two-stage risk prediction process of obstetric and perinatal complications, providing differentiated, pathogenetically grounded treatment based on the woman's risk level and clinical condition. The high effectiveness of the proposed treatment complex has been proved.

Following the recommended treatment, HIV progression indicators improved. The detoxifying effect of therapy is evidenced by the reduction in bilirubin levels and cytolytic markers. There is an improvement in the intestinal microecology and vaginal microbial landscape. The incidence of pregnancy complications decreased by 23.3%: the rate of placental insufficiency and preeclampsia decreased almost twice, and, accordingly, the rate of fetal distress

decreased to 20%. The condition of newborns significantly improved: 60.0% of babies were born in satisfactory condition (without asphyxia), and the incidence of jaundice, hemorrhagic, and gastrointestinal syndromes was 1.5 – 2 times lower. The frequency of life-threatening syndromes decreased twice: neurological syndrome (decreased to 23.3%) and craniosynostosis syndrome (decreased to 16.7%). Thanks to the use of specific immunoglobulins, the frequency of intrauterine herpes virus infection decreased by three times.

The safety and high effectiveness of the proposed complex for management of HIV-infected pregnant women with herpes virus infection allow us to recommend it for implementation in maternity clinics and auxiliary medical institutions.

References:

1. Borak V. P., Romaniuk L. B., Borak V. T., Kravets N. Ia. (2016). Do pytannya pro herpetychnu infektsiyu yak aktual'nu problemu s'ohodennya [On the herpes infection by topical issue today]. Aktualna infektohoiia, no. 2 (11), pp. 53–58.
2. Holokhvastova E., Tsarenko S., Lytvynova N., Perehudova A., Leonova T., Shakhlydian V., Olshanskyi A., Mazus A. (2012). Spektr i chastota opportunisticheskikh zaboolevaniy u bolnykh VICH-infektsiy [The range and frequency of opportunistic infections in HIV-infected patients]. Vrach, no. 6, pp. 26–30.
3. Vasil'ev V. V. (2013). Vrozhdeniye infektsii v praktike semeynogo vracha [Congenital infection in the practice of family doctors]. Rossiyskiy semeynyy vrach. 17 (1): 16–27.
4. Popova A. F., Pastushenkov V. L. (2010). Klinicheskie i laboratornye aspektyi gerpeticheskoy infektsii u patsientok s nevyinashivaniem beremennosti [Clinical and laboratory aspects of herpes infection in patients with miscarriage]. Zhurnal akusherstva y zhenskikh boleznei, no. 6, pp. 58–68.
5. Isakov V. A., Isakov D. V. (2014). Patogenez i lechenie sotsialno znachimykh virusnykh urogenitalnykh infektsiy (gerpesa i papillomavirusnoy infektsii) [Pathogenesis and treatment of socially significant viral urogenital infections (herpes and papillomavirus infection)]. Klin. farmakologiya i terapiya, no. 23 (1), pp. 7–13.
6. Nigro G., Adler S. P., Parruti G. (2012). Immunoglobulin therapy of fetal cytomegalovirus infection occurring in the first half of pregnancy – a case-control study of the outcome in children. J. Infect. Dis, no. 205(2), pp. 215–227.
7. Kuz'min V. N. (2014). Sovremennyy vzglyad na klinicheskoe techenie, diagnostiku i terapiyu genitalnogo gerpesa u zhenshin [The modern view of the clinical course, diagnosis and treatment of genital herpes in women]. Consilium Medicum, no. 06, pp. 55–60.
8. Belyakov N. A., Rassokhin V. V., Stepanova E. V. VICH-infektsiya, vtorichnyye i soputstvuyushchie zabolevaniya [HIV infection, secondary and comorbidities]. Ed. Tematicheskii arkhiv. (2014). Saint Petersburg, Baltiyskiy meditsinskiy obrazovatel'nyy tsentr, p. 368.
9. Anoshyna T. M. (2015) Perinatalni nasliddki u VIL-Inflkovanih vagltnih z herpesvirusnoy Infektsieyu [Perinatal outcomes in HIV-infected pregnant women with herpes virus infection]. Sovremennaya pediatriya, no. 6 (70), pp. 110–112.
10. Yatsenko D. S. (2015). Techenie beremennosti, rodov i sostoyanie novorozhdennykh u zhenshin, infitsirovannykh VICH infektsiey [The course of pregnancy, childbirth and neonatal status in women with HIV infection]. BМИK, no. 5, p. 48.

References: Kaminskyi V. V., Anoshyna T. M., Kolomiichenko T. V. *Management of Pregnancy in the Association of HIV and Herpes Virus Infection // "Reproductive Health Eastern Europe". – 2016. – Volume 6, No. 6. – pp. 725–732.*

6.6. APPLICATION OF PROTEFLAZID® FOR PREGNANT WITH ASSOCIATED TOXOPLASMIC, HERPETIC, CYTOMEGALOVIRAL AND EPSTEIN-BARR-VIRAL INFECTION

I. V. Lipkovska, V. H. Marychereda, I. L. Holovatiuk, O. P. Holovatiuk

Abstract

Under a supervision was 300 pregnant with the latent form of toxoplasmosis, that is associated with herpetic, cytomegaloviral and Epstein-Barr-viral infection. The syndrome of child loss in former pregnancies is marked at 45 pregnant (15%), premature births – at 30 pregnant (10%). PROTEFLAZID® accepted 192 pregnant in a term 12-37 weeks of gestation, after authentications of CMV, HSV and EBV by the methods of IFA and PCR on a background an asymptomatic toxoplasmosis. 218 Application of PROTEFLAZID® during 3 months in the term of gestation is 12-37 weeks normalized by the index of avidity of anti-IgG CMV, anti-IgG HSV accordingly in 97,0% pregnant, hinders to forming in blood of proof seroconversions of anti-IgG in 41% pregnant with high antibodygenesis anti-IgG CMV, anti-IgG HSV I to the type, anti-IgG EBV on a background an asymptomatic toxoplasmosis. At the proof subzero index of avidity of anti-IgG CMV, HSV can be the recommended repeated courses of PROTEFLAZID® reception for a term from 1 to 3 months Taking into account the high degree of toxicological safety of PROTEFLAZID® and absence of refracterity at the use, beginning from 12 weeks of pregnancy its can be recommended with the purpose of normalization of immunological type for pregnant with the united toxoplasmosis with viral infection.

Key words: toxoplasmosis, cytomegaly, herpetic infection, EBV-infection, pregnancy, antibodies, PCR, PROTEFLAZID®.

Introduction

At present, various infections play an important role in obstetric and gynecological pathology [1, 2, 4, 8, 14, 21–23, 32]. Some of them (cytomegaly, toxoplasmosis, infectious mononucleosis) are transmitted transplacentally and result in congenital pathologies, early childhood mortality, or disability [5–7, 11, 15, 16, 25–29]. The risk of fetal infection increases up to 60% in the presence of cytomegalovirus infection in pregnant women [8, 21, 31], and up to 40–50% in cases of primary herpes infection [3, 18]. In 40% of children diagnosed with hydrocephalus, the presence of EBV has been confirmed [9].

Various DNA-containing viral and viral-parasitic associations in biological fluids (CMV+EBV, CMV+T. gondii, EBV+Parvovirus, etc.) have been confirmed in 2 out of every 10 newborns [9]. At the same time, the presence of toxoplasmosis infection associated with various viral pathogens exerts an influence throughout pregnancy. In particular, the presence in the blood of a high level

of anti-IgG antibodies to *T. gondii* and persistent IgG antibody seroconversion to *T. gondii* during pregnancy, combined with high antibody titers of anti-IgG CMV, anti-IgG HSV-1, and anti-IgG EBV in 40% of pregnant women with toxoplasmosis infection, increases the risk of miscarriage. The presence of a negative avidity index of anti-CMV IgG and anti-NS IgG against the background of asymptomatic toxoplasmosis infection in pregnant women leads to the development of oligohydramnios, polyhydramnios, and placental dysfunction.

The difficulties in management tactics of these pregnant patients are associated with the following factors:

1. The absence of clear clinical symptoms of the infectious process during pregnancy, and the difficulty of comprehensive diagnostics for toxoplasmosis infection (anti-toxo IgA + anti-toxo IgM + anti-toxo IgG + anti-toxo IgG avidity + PCR) against the background of unspecified cytomegalovirus (anti-CMV IgM, IgG + anti-CMV IgG avidity + PCR), and herpes infection (anti-HSV IgM + anti-HSV IgG + anti-HSV IgG avidity + PCR); anti-EBV to early capsid antigen, anti-IgM, anti-IgG, PCR of blood, saliva, and urine (qualitative and quantitative analysis to determine viral load in order to differentiate between asymptomatic carriers and infected women) [9, 11–13, 15, 21, 22, 25, 27–32].

2. The absence of practical guidelines for managing pregnant women with concurrent toxoplasmosis, herpes, cytomegalovirus, and EBV infections in accordance with ICD-10, and in the obstetric and gynecological protocols related to “Perinatal infections” (Order of the Ministry of Health of Ukraine No. 906 dated 27.12.2006 “On Approval of the Clinical Protocol for Obstetric Care ‘Perinatal Infections’”).

In this regard, we conducted an extended diagnostic evaluation of toxoplasmosis in pregnant women with concurrent herpesvirus, cytomegalovirus, and EBV infections in accordance with ICD-10, using WHO standards.

Materials and Methods

We observed 300 pregnant women with a latent form of toxoplasmosis associated with herpesvirus, cytomegalovirus, and EBV infections. The average age of the women studied was 22.3 ± 4.5 years. 72% of those examined women reported contacts with animals, and 90% reported consumption of thermally unprocessed food.

According to the obstetric and gynecological history of mothers, fetal loss syndrome in previous pregnancies was noted in 45 pregnant women (15%), and preterm labor in 30 pregnant women (10%).

The final diagnosis of “Toxoplasmosis infection” was established in accordance with ICD-2010 (unspecified toxoplasmosis, toxoplasmosis infection caused by the parasite *T. gondii*, toxoplasmosis with involvement of other organs) based on the data from epidemiological history, the presence or absence of clinical symptoms, physical examination, general clinical tests, special instrumental methods (ultrasound examination), and extended toxoplasmosis diagnostics according to WHO standards (detection in blood of class A, M, and G antibodies to *T. gondii* by ELISA method, determination of IgG antibody avidity index, PCR diagnostics of biological fluids).

The final diagnoses of “Cytomegalovirus infection,” “Herpetic infection,” and “Viral infection caused by Epstein–Barr virus” were established in accordance with ICD-2010 based on epidemiological history, the presence or absence of clinical symptoms, physical examination, general clinical tests, special instrumental methods (ultrasound examination and extended blood diagnostics – detection in blood of class M and G antibodies to CMV, class A, M, and G antibodies to HSV by ELISA, anti-EBV antibodies to early capsid antigen of EBV, anti-IgM, anti-IgG

EBV, determination of IgG antibody avidity index for CMV and HSV, PCR diagnostics of saliva and blood).

For statistical reliability, all studies were conducted using uniform test systems. For the mathematical processing of the obtained results, the software Statistika 3.0–6.0 was used.

A special risk group included patients with detected class A and M antibodies to HSV 1 and 2 types (n=5), anti-IgM CMV (n=3), EBV infection in the acute phase (n=2), positive PCR saliva test (EBV, n=12), and reduced or negative avidity index for herpetic (n=42) and cytomegalovirus infections (n=34), against the background of asymptomatic toxoplasmosis infection, who administered medicinal product PROTEFLAZID® (drops) during pregnancy.

PROTEFLAZID® (drops) is registered in Ukraine as an antiviral and immunomodulating herbal medicinal product (flavonoid glycosides derived from wild grasses *Deschampsia caespitosa* L. and *Calamagrostis epigeios* L.). The primary effect of the medicinal product is its ability to inhibit virus-specific enzymes – DNA polymerase, thymidine kinase, and reverse transcriptase – in cells infected with viruses, leading to the blockade of viral DNA replication and interruption of viral reproduction. In addition, PROTEFLAZID®, by acting on the endogenous interferon system, stimulates the non-specific activity of the macrophage system, subsequently modulating the state of cellular immunity and initiating reparative and regenerative processes.

The medicinal product normalizes the synthesis of endogenous alpha- and gamma-interferons without inducing refractoriness of the immune system, which indicates its immunological safety [1, 3, 8, 10, 17, 18, 20, 24].

Experimental studies have shown that PROTEFLAZID® does not exhibit carcinogenic, embryotoxic, or teratogenic effects and meets the toxicological safety requirements for use during pregnancy and breastfeeding [3, 10, 17, 24].

PROTEFLAZID® has been successfully used in patients for the treatment of cytomegalovirus infection [18], infectious mononucleosis [20], herpetic infection in pregnant women [3, 17].

It has been included in the protocols of the Ministry of Health of Ukraine “Infectious Diseases” for the treatment of patients with herpetic and cytomegalovirus infections.

PROTEFLAZID® was prescribed for patients during the gestational period of 12–37 weeks of pregnancy orally during the 1st week – 7 drops 2 times a day; during the 2nd and 3rd weeks – 15 drops 2 times a day; during the 4th week and further for 1–2 months – 12 drops 2 times a day.

Practically, there are no analogues of the use of PROTEFLAZID® in early pregnancy period on the territory of the CIS.

Study objective

To study the peculiarities of the course of toxoplasmosis infection associated with herpetic, cytomegalovirus, and EBV infections according to ICD-10 and WHO standards. To apply extended blood diagnostics using ELISA methods (anti-IgA, anti-IgM, anti-IgG + avidity index, anti-IgG), PCR – quantitative and qualitative analysis. To use PROTEFLAZID® (drops) in the therapy of pregnant women with clinical signs of herpetic, cytomegalovirus infections, and Epstein–Barr virus (EBV) infection on the background of asymptomatic toxoplasmosis infection in individuals with an altered immunological profile, identified and confirmed by ELISA and PCR diagnostics of blood and other biological fluids, in the absence of apparent clinical symptoms.

Study results and their discussion

Most often, toxoplasmosis infection in pregnant women was combined with unspecified herpetic infection (n=300), unspecified cytomegalovirus infection (n=300). Less frequently – with unspecified infectious mononucleosis (n=110 – 36.0%). Clinical manifestations of herpes were

identified in 5 pregnant women, of cytomegaly – in 2 women. Clinical signs of EBV infection manifestation (fever, cervical lymphadenitis) were noted in only 2 patients.

In 98% of women, toxoplasmosis infection proceeded without any clinical manifestations. Subfebrile temperature and lymphadenitis were observed only in very rare cases.

A total of 192 pregnant women took PROTEFLAZID® at 12–37 weeks of gestation, after the identification of CMV, HSV, and EBV by ELISA and PCR methods, against the background of asymptomatic toxoplasmosis infection.

In 5 pregnant women using PROTEFLAZID® with detected class M antibodies to herpes simplex virus type 1 and clinical signs of labial herpes, rapid elimination of acute-phase IgA antibodies (n=2) and IgM antibodies (n=5) from the blood was observed. The following results were observed in 5 pregnant patients: IgM values (1.12 ± 0.58 extinction units before PROTEFLAZID® administration and a negative result on the 10th day of medicinal product administration).

A trend toward elimination of acute-phase anti-CMV IgM in the blood of 3 pregnant women while taking the medicinal product PROTEFLAZID® was accompanied by normalization of the level of anti-IgM CMV from (1.80 ± 0.81 optical density units to control values of 0.31 ± 0.05 ; $p > 0.05$). PCR diagnostics of blood, saliva, and urine for CMV yielded negative results. In 2 pregnant women with signs of subfebrile temperature and cervical lymphadenitis (saliva PCR EBV “+”), with detected anti-EBV antibodies to the early capsid antigen and anti-EBV IgM, by the 14th day of taking the medicinal product, normalization of antibody levels of anti-EBV to the early capsid antigen from 0.64 to 0.23 ± 0.09 extinction units (n=2), and decreasing anti-EBV IgM levels from 1.44 ± 4.12 to 0.76 ± 0.02 optical density units ($p < 0.05$). On the 7th day of taking PROTEFLAZID®, patients general conditions improved, body temperature returned to normal, symptoms of lymphadenitis subsided, and by the 14th day of taking PROTEFLAZID®, elimination of EBV from saliva was confirmed by PCR diagnostics.

Taking the medicinal product PROTEFLAZID® (drops) during one month promoted elimination of EBV from saliva in 7 out of 10 pregnant women. In the remaining 3 pregnant women, a decrease in viral load was noted. Continued therapy using the medicinal product PROTEFLAZID®, 12 drops twice a day for the following month, led to complete elimination of the virus from saliva.

Table 1. Dynamics of restoration of the negative avidity index of anti-IgG HSV of types 1 and 2, anti-CMV IgG during treatment with the medicinal product PROTEFLAZID® (n=76)

Indicator	At the treatment start		After 4 weeks		After 8 weeks		After 12 weeks	
	Absolute value	%	Absolute value	%	Absolute value	%	Absolute value	%
Anti-HSV 1-2 «->»	46	100,0	30	65	15	33	3	6
Anti CMV «->»	30	100,0	15	50	10	33	4	13

From the data presented in Table 1, it is evident that during treatment with the medicinal product PROTEFLAZID®, 15 drops twice a day for one month, a negative avidity index of anti-HSV IgG was diagnosed in 65% of pregnant women, after 8 weeks of intake – in 33%, and after 12 weeks – in 6%. The effectiveness, based on the normalization of the avidity index of anti-HSV IgG in the blood of pregnant women, was 97.0%.

In patients with the negative avidity index of anti-CMV IgG during treatment with the medicinal product PROTEFLAZID®, 15 drops twice a day for one month, a negative avidity index of

anti-HSV IgG was diagnosed in 50.0% of pregnant women, after 8 weeks of intake – in 33%, and after 12 weeks – in 13%. The effectiveness, based on the normalization of the avidity index of anti-HSV IgG in the blood of pregnant women, was 94.8%.

In 3 patients, low avidity of class IgG antibodies to herpes types 1 and 2 persisted even after 12 weeks of using the medicinal product PROTEFLAZID®.

A negative avidity index of anti-IgG CMV was diagnosed in the blood of 4 pregnant women after 12 weeks of taking the medicinal product PROTEFLAZID®.

Administration of the medicinal product PROTEFLAZID® in 76 pregnant women with negative avidity of anti-HSV IgG (n=46) and anti-CMV IgG “–” for 3 months leads to normalization of the anti-IgG avidity index. Accordingly, after treatment the following values were reached: (58.6±2.2)% compared to (60.2±2.3)% in the control group among pregnant women with a negative avidity index to herpes type 1 before treatment, and (57.2±2.1)% compared to (59.4±3.2)% (in the control group) in patients with negative avidity of anti-CMV IgG before using the medicinal product PROTEFLAZID® (p>0.05, Table 2).

Table 2. Dynamics of changing the avidity index (M±m)% (anti-IgG CMV, anti-IgG HSV types 1 and 2) in pregnant women with associative toxoplasma infection during treatment with the medicinal product PROTEFLAZID®

	Control	Before the treatment start	Proteflazid (n=76)	P
HSV (n=46)	60,2±2,34	negative	58,6±2,2	>0,05
CMV (n=30)	59,4±3,2	negative	57,2±2,1	>0,05

A correlation was found in pregnant women with altered avidity index (anti-IgG CMV, anti-IgG HSV of types 1 and 2) – between the increase in the level of T-helper inducers (CD4+, CD8+) and the number of T-cytotoxic cells (CD4+, CD88+) – (0.76±0.63). At the same time, the level of T-helper inducers (CD4+, CD8+) during a one-month administration of the medicinal product PROTEFLAZID® decreased, but remained statistically different from the level in healthy individuals – (40.23±2.39), compared to (31.43±3.56)% (p<0.05), and differed only slightly from the level of T-helper inducers (CD4+, CD8+) in pregnant women who received Viferon as part of combination therapy – (42.40±3.77)% (p>0.05).

From the data presented in Table 3, it is evident that administration of the medicinal product PROTEFLAZID® by 124 pregnant women with high antibody production of anti-CMV IgG, anti-HSV IgG, anti-EBV IgG against the background of toxoplasmosis infection, prevents further increase of IgG class antibodies during pregnancy. Although the reduction in anti-IgG (CMV, HSV, EBV) levels was insignificant, this contributed to a reduced risk of pregnancy loss in 41% of pregnant women.

At the same time, during the treatment with PROTEFLAZID®, the risk of pregnancy complications was reduced: polyhydramnios and oligohydramnios were diagnosed in only 3% of pregnant women with toxoplasmosis infection.

In 10 pregnant women, even after delivery, a zero-avidity index of anti-CMV IgG and anti-HSV IgG was preserved in the blood. All of these women showed no clinical signs of herpes or cytomegalovirus infection.

At the same time, in the blood of the newborns, no antibodies of class A or M to herpes infection, no anti-IgM CMV, or anti-IgM to early capsid antigen of EBV were detected. During PCR diagnostics of blood for *T. gondii*, CMV, HSV types 1 and 2, and Epstein-Barr virus, no pathogens were detected.

Table 3. Dynamics of antibody response (anti-CMV IgG, HSV IgG, anti-EBV IgG in IU/ml) in pregnant women with toxoplasmosis infection during treatment with the medicinal product PROTEFLAZID®

ELISA	Number of pregnant women		Control M±m	Received PROTEFLAZID® (before treatment)	Received PROTEFLAZID® (after treatment)	Did not receive PROTEFLAZID®	p ₁	p ₂	p ₃	p ₄
	abs. value	%								
anti-CMV IgG	40	13,0	0,22 ±0,06	1,90±0,12	1,60±1,10	2,40±0,19	<0,05	<0,05	<0,05	>0,05
anti-HSV IgG	54	18,0	0,26 ±0,06	2,11±0,02	1,90±0,04	2,44±0,03	<0,05	<0,05	<0,05	>0,05
anti-EBV IgG	30	10,0	0,32 ±0,04	2,43±0,05	2,00±0,01	2,60±0,04	<0,05	<0,05	<0,05	>0,05

Notes:

p₁ – statistical significance of the difference between the anti-IgG level in the control group and the anti-IgG level in the observed group before treatment;

p₂ – statistical significance of the difference between the anti-IgG level in the control group and the anti-IgG level in the observed group after treatment;

p₃ – statistical significance of the difference between the anti-IgG level in the control group and the anti-IgG level in pregnant women who did not receive PROTEFLAZID® during pregnancy;

p₄ – statistical significance of the difference between the anti-IgG level before and after PROTEFLAZID® administration.

Conclusions

1. The use of the medicinal product PROTEFLAZID® (drops) for 3 months during the gestational period of 12–37 weeks normalizes the avidity index of anti-IgG CMV and anti-IgG HSV respectively in 97% of pregnant women, and prevents the formation of persistent anti-IgG seroconversions in the blood in 41% of pregnant women with high antibody production of anti-IgG CMV, anti-IgG HSV type 1, and anti-IgG EBV in the presence of asymptomatic toxoplasmosis infection.

2. In cases of persistently low avidity index of anti-IgG CMV and HSV, repeated courses of PROTEFLAZID® intake lasting from 1 to 3 months may be recommended.

3. The medicinal product PROTEFLAZID® promotes rapid elimination from the blood of acute-phase markers of cytomegalovirus, herpes, and EBV infections, as well as elimination of EBV from the saliva of pregnant women with co-existing toxoplasmosis infection, thus preventing manifestation of the infectious process during pregnancy.

4. In babies born to infected mothers with associated toxoplasmosis infection (CMV, HSV, EBV) who received the medicinal product PROTEFLAZID® during pregnancy, PCR diagnostics of blood detected no CMV, HSV, or EBV.

5. Taking into account the high level of toxicological safety of the medicinal product PROTEFLAZID® and the absence of refractoriness when taken orally starting from the 12th week of pregnancy, it may be recommended for the purpose of normalizing the immunological profile in pregnant women with combined toxoplasmosis and viral infections.

References:

1. Азімова О. І. До питання профілактики рецидиву генітального герпесу у вагітних з антенатальною загибеллю плода в анамнезі / О. І. Азімова, С. Н. Султанов, Ф. П. Нішанова // *Здоров'я жінки*. – 2011. – №2 (58). – С. 238–240.
2. Актуальні аспекти вроджених інфекцій у Росії / Ю. В. Лобзін, В. В. Васильєв [та ін.] // *Журнал інфектології*. – 2010. – №2. – С. 14–24.
3. Бенюк В. О. Периконцепційна профілактика і лікування герпесвірусної інфекції у вагітних з метаболічним синдромом / В. О. Бенюк, О. А. Диндар, П. О. Лихолай // *Здоров'я жінки*. – 2012. – №9 (75). – С. 116–120.
4. Васильєв В. В., Васильєва І. С. Токсоплазмоз / В. В. Васильєв, І. С. Васильєва // *Вибрані питання терапії інфекційних хворих*. – За ред. Ю. В. Лобзіна. – СП Фоліант, 2005. – С. 581–596.
5. Васильєв В. В. Прогноз порушень репродуктивної функції у жінок, хворих на хронічний токсоплазмоз / В. В. Васильєв, М. М. Кутарьова // *Сибірський медичний журнал*. – 2008. – №7. – С. 59–61.
6. Возіанова Ж. І. Інфекційні і паразитарні хвороби: В 3 т. / Ж. І. Возіанова. – К.: Здоров'я, 2000. – Т. 2. – С. 532–535.
7. Глуховець Б. І. Кого і що приховує «синдром раптової смерті немовлят» / Б. І. Глуховець, Н. Г. Глуховець // *Праці V з'їзду Російського товариства дитячих патологій (Санкт-Петербург, 21–22 травня 2012 року)*. – СПб. – С. 117–124.
8. Глей А. І. Цитомегаловірусна інфекція / А. І. Глей // *Therapia*. – 2007. – №4. – С. 27–28.
9. Інфекційна перинатальна патологія: розробка стратегії діагностики та клініко-лабораторного моніторингу / Т. І. Довгих, С. В. Барінов, Є. Г. Проданчук [та ін.] // *Медичний алфавіт. Сучасні лабораторії*. – 2011. – №1. – С. 17–20.
10. Інструкція для медичного застосування препарату Протефлазид® (Затверджено наказом Міністерства охорони здоров'я України №113 від 01.03.2011. Реєстраційне свідоцтво №UA/4220/01/01).
11. Корюкіна І. П. Синдром раптової смерті в структурі дитячої смертності Пермського краю / І. П. Корюкіна, Л. В. Софронова, Л. П. Санакоїва // *Пермський медичний журнал*. – 2011. – Т. XXVIII, №6. – С. 137–141.
12. Кудін А. П. Ця «нешкідлива» вірус Епштейна-Барр-інфекція. Частина 1. Характеристика збудника. Реакція імунної системи на вірус / А. П. Кудін // *Мед. новини*. – 2006. – №7. – С. 14–22. 216
13. Керівництво та атлас з інфекційних та паразитарних хвороб людини / За ред. Ю. В. Лобзіна, С. С. Козлова. – СПб. : Фенікс, 2008. – 932 с.
14. Львова І. І. Ефективність управління ризиком TORCH-інфекцій у новонароджених дітей у Пермському краї / І. І. Львова, І. Б. Яковлев, М. Ю. Новіков // *Дитячі інфекції*. – 2011. – Т. 10, №1. – С. 18–21.
15. Марков І. С. Діагностика та лікування герпетичних інфекцій та токсоплазмозу / І. С. Марков. – К.: АртЕк, 2002. – 192 с.
16. Мельник М. М. Інфекційні захворювання. Керівництво для лікарів / За ред. В. І. Покровського, М. М. Мельника, П. К. Коровицького, А. І. Григорашенка [та ін.]. – М.: Медицина, 1996. – 528 с.
17. Нагорна В. Ф. Оцінка ефективності препарату Протефлазид® у схемах супресивної терапії герпетичної інфекції у пацієнтів зі звичним невиношуванням вагітності / В. Ф. Нагорна, С. В. Николаєва // *Репродуктивне здоров'я жінки*. – 2006. – №1 (25). – С. 88–90.
18. Прилуцький О. С. Застосування індукторів інтерферону з метою зниження вірусу виділення при цитомегаловірусній інфекції / О. С. Прилуцький, Д. А. Лісниченко, Е. А. Майлян // *Імунологія та алергологія*. – 2004. – №2. – С. 7–8.

19. Раціональна лабораторна діагностика герпес-перинатальних інфекцій – основа клінічної ефективності / Т. І. Довгих, С. В. Барінов, Т. В. Кодцина, Е. М. Овчиннікова // Медичний алфавіт. – 2012. – Т. 1, №2. – С. 12–14.
20. Крамарьов С. О. Досвід застосування препарату Флавозід® при Епштейна-Барр-вірусній інфекції у дітей / С. О. Крамарьов, О. В. Виговська // Сучасна педіатрія. – 2011. – №5 (39). – С. 1–5.
21. Смирнова А. І. Роль цитомегаловірусів в акушерській практиці та неонатології / А. І. Смирнова, С. В. Россіхіна, Н. С. Дюпіна // Вятський медичний вісник. – 2010. – №4. – С. 41–47.
22. TORCH-інфекції в акушерстві та неонатології / За ред. Т. К. Знам'янської. – К., 2008. – 200 с.
23. Тартаковська Р. Вроджена та набута цитомегаловірусна інфекція у дітей / Р. Тартаковська // Відомості Дагестанського державного педагогічного університету, природничі та точні науки. – 2012. – №2. – С. 79–84.
24. Чекман І. С. Вивчення безпеки препарату Протефлазид® за показниками місцево-подразнюючого алергічного, ембріотоксичного та мутагенного дій / І. С. Чекман // Національний медичний університет. – Київ, 1997 (за даними компанії).
25. Diagnosis of congenital infection / A. S. Martinez, L. A. Martinez, P. M. Teatino, J. Rodriguez-Grander // Enferm. Infecc. Microbiol. Clin. – 2011. – V. 5. – P. 15–20.
26. Karczewski G. Diagnostic problems with congenital toxoplasmosis / G. Karczewski, E. Gołab // Przegl. Epidemiol. – 2011. – N 3 (65). – P. 451–454.
27. Epidemiological and clinicoimmunological monitoring of toxoplasmosis in the Omsk Region / T. I. Dolgikh, N. S. Zapariĭ, T. V. Kadtsyna, A. V. Kalitin // Med. Parazitol. (Mosk). – 2008. – №1. – P. 19–22.
28. European multicenter study of the LIAISON automated diagnostic system for determination of *Toxoplasma gondii* – specific immunoglobulin G (IgG) and IgM and the IgG avidity index / E. Petersen, M. Y. Borobio, E. Guy [et al.] // J. Clin. Microbiol. – 2005. – V. 43 (4). – P. 1570–1574.
29. First Colombian multicentric newborn screening for congenital toxoplasmosis / J. E. Gómez-Marin, A. de-la-Torre [and others] // PLoS Negl. Trop. Dis. – 2011. – N 5. – P. 1195. 217
30. Human peripheral blood and bone marrow Epstein-Barr virus-specific T-cell repertoire in latent infection reveals distinct memory T-cell subsets / M. Guerreiro, I. K. Na, A. Letsch [et al.] // Eur. J. Immunol. – 2010. – Vol. 40, N 6. – P. 1566–1576.
31. Laboratory testing for cytomegalovirus among pregnant women in the United States: a retrospective study using administrative claims data / J. Leung, M. G. Cannon, S. D. Gross, S. R. Bialek // BMC Infect. Dis. – 2012. – N 12 (1). – P. 334.
32. *Toxoplasma gondii* infection in pregnant women in China / X. J. Gao, Z. J. Zhao [and others] // Parasitology. – 2012. – N 139 (2). – P. 139–147.

Reference: *Lipkovska I. V., Marichereda V. H., Holovatiuk I. L., Holovatiuk O. P. Application of Proteflazid® for Pregnant with Associated Toxoplasmic, Herpetic, Cytomegaloviral and Epstein-Barr-Viral Infection. / Ministry of Defense of Ukraine, Military Medical Department, Ukrainian Military Medical Academy. – Problems of Military Health Protection // Collection of Scientific Papers of the Ukrainian Military Medical Academy. – Issue No. 42, Volume 2. – Edited by Professor V.Ya. Bilyi, Kyiv. – 2014, – pp. 207–218.*



Section 7

7.1. PROTEFLAZID®: CLINICAL AND ECONOMIC SUBSTANTIATION FOR USE IN THERAPY OF HERPES INFECTION

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Abstract

Epidemiological data indicate the widespread dissemination and constant dynamics of the incidence rate of herpesvirus infections due to a variety of forms and transmission paths of the virus, highly contagious and life-long persistence of the herpes virus in the body of the infected persons and the lack of effectiveness of existing treatments. Acyclic guanosine derivatives are the main group of antiviral drugs traditionally used for the causal treatment of herpes infections. They have a number of disadvantages, in particular formation of the virus resistance in a long-term use, the need to involve immunotherapy, as well as the high cost of treatment. The accumulated clinical experience with the original Ukrainian drug PROTEFLAZID® (drops) in treating viral infections, including HHV- infections, shows its high clinical efficacy and safety. A relative estimation of the course treatment costs of herpes-induced infections using antiviral drugs has demonstrated the economic expediency of PROTEFLAZID® with the purpose of treatment and maintenance therapy. PROTEFLAZID® takes the medium sized niche among other antiherpetic drugs. In addition, due to the multi-vector nature of its pharmacodynamic effects (direct antiviral, immunomodulating, interferon-stimulating, antioxidant, apoptosis-modulating, etc.) PROTEFLAZID® allows to exclude antioxidants, immunomodulators from the antiherpetic therapy scheme, or to adjust their use, and it can significantly reduce the cost per a course of treatment.

Key words: herpes infections; antiviral drugs; acyclic nucleosides; PROTEFLAZID®; clinical efficiency; cost of treatment.

Вступ

Among the viral diseases herpesvirus infections are the most commonly encountered due to the widespread dissemination of viruses, a variety of transmission paths of the virus, highly contagious and life-long persistence of the herpes virus in the body of the infected persons and the lack of effectiveness of existing treatments [24]. The incidence of herpes infection is difficult to quantify, partly because of unrecognized or asymptomatic course of the disease. Nevertheless, the incidence of herpes infections in the North American and European populations is between 5 and 24 per 100 people per year [29]. Herpes viruses (HHV) are widespread in the industrialized countries. According to the epidemiological studies conducted in Europe, the USA and Canada, 95% of immunocompetent persons aged 50 years and older are seropositive for varicella zoster virus, and therefore, at risk of developing herpes zoster disease [25]. It has been also shown that the average age of adults infected with herpes zoster

is 59.4 years, and 68% of cases occur in people aged 50 years and older. The incidence in women was significantly higher than that in men [32]. Up to 45% of the population between the ages of 6 months to 79 years is seropositive for cytomegalovirus infections [27].

Getting into the human body the herpes virus stays in it for life, staying in a latent or persistent state and undergoing reactivation in favourable conditions. In this way a chronic, latent, recurrent or progressive infection and its complications is formed; it may cause temporary disability, the development of cancer and other complications, as well as disability or death [5]. The herpes infection disease leads to significant economic losses associated with both the incapacity of the patient, and difficulties and the duration of treatment of the disease [11]. This is due, first of all, to the complexity of the strategy of parasitism and opportunistic properties of these pathogens, multiple organ lesions, the presence of numerous complications and multi-vector nature of some lesions [5]. It is known that the main differences of the replicative cycle of the herpes family viruses are associated with the more complex structure of the genome compared to other DNA-containing viruses. Thus, these viral agent genes, which encode the protein structure, make up only 15% of the DNA since most of the genome is occupied by genes, which are responsible for the synthesis of enzymes and regulatory proteins. This makes it possible to show the herpes viruses parasitizing action, including the possibility of latent, persistent and reactivated state in the infected organism [5].

Due to the complexity of herpes treatment the cost of antiherpetic therapy, which is determined by the cost of drugs and duration of their use for the treatment of the disease and its complications and/or prophylactic use, is of significant importance. For example, the cost of Herpes Zoster (Varicella zoster – VZV, HHV III) therapy in France is 4206 € and more per a relapse; direct costs associated with the treatment of postherpetic neu ralgia in Sweden are up to € 939, or more than 19 million € per 100 000 population [23, 28].

Over the past decade a significant number of antiherpetic drugs, which are different in origin (natural, synthetic), the structure and properties, has been introduced in clinical practice [17]. Herbal drugs have been also applied in the treatment of herpes infections. Among them Proteflazid® – the Ukrainian original drug with the antiviral effect due to flavonoid glycosides containing in wild grasses of *Deschampsia caespitosa* L. and *Calamagrostis epigeios* L. should be mentioned [1, 6, 26]. Thanks to the direct antiviral action, as well as the ability of biologically active substances of Proteflazid to activate the humoral and cellular immunity and induce the synthesis of endogenous interferon, the drug has shown a high clinical efficacy in the treatment of viral infections, including those caused by HHV.

Table 1 shows the main drugs traditionally used to treat herpes infections. According to the modern requirements the ideal antiviral agent should have a wide spectrum of the antiherpetic action (the activity against herpes viruses I and II types, Varicella zoster (VZV – HHV III), Epstein- Barr (EBV – HHV IV), cytomegalovirus (CMV – HHV V) and etc.), low toxicity, the absence of adverse effects on the immune system, a good ability to penetrate a cell, act on a virus with a high selectivity, have the minimal general cytotoxicity, do not induce resistance and do not cumulate in the organism. The pharmacokinetic characteristics of antiherpetic drugs such as the rapid achievement of the infected cells, the prolonged presence of the drug in the active form, as well as ease of the dosing regimen (1-2 times daily), are also important. Specific requirements are imposed to safety – an antiherpetic drug must be safe in short-term use (especially in the elderly, pregnant women and children, including newborns), and in a long-term suppressive therapy.

Table 1. Drugs used for treatment of herpes infections

Parameter		PROTE-FLAZID®	Acyclovir	Valacyclovir	Ganciclovir	Famciclovir
Bioavailability		High, more than 80%	Low, 8-10%	Moderate, 50-60%	Low, up to 10%	High, up to 77%
T ½, h		5-9	2-3	≥3	3-4	2-2,8
Pharmacodynamic effects		Antiviral, immunostimulating, antioxidant, apoptosismodulating	Antiviral	Antiviral	Antiviral	Antiviral
Indications for diseases caused by viruses:	HSV-I	+	+	+	–	–
	HSV-II;	+	+	+	–	+
	HHV-III – VZV	+	+	+	–	+
	HHV-IV – EBV	+	–	–	–	–
	HHV-V – CMV	+	–	+	+	–
	HHV-VI	–	–	–	–	–
	HHV-VII	–	–	–	–	–
	HHV-VIII	–	–	–	–	–
The presence of resistance		–	+	+	+	+
Children		Since birth	Over 2 years	Over 12 years	Over 12 years	Over 12 years
Pregnancy / lactation		Permitted	Undesirable / prohibited	Undesirable	Prohibited	Undesirable
Dosage form		Drops	Tablets	Tablets	Tablets	Tablets

As a rule, all antiviral drugs have low toxicity – their LD50 value ranges from 2.000 mg/kg to 20.000 mg/kg after a single intragastric introduction in animals [19]. However, a lot of them have a wide spectrum of undesirable side reactions, in particular, ganciclovir possesses significant toxic effects, and the expressed hematotoxicity (thrombocytopenia, neutropenia, etc.) is inherent to it. It should be also noted that because of the high toxicity and mutagenic activity of antiviral drugs the effective methods of contraception should be used by patients of the reproductive age during the treatment and up to 90 days after the end of therapy [21]. To some extent it narrows the niche of the drug use (severe cases of cytomegalovirus infection (CMV), infections in transplantation, patients with HIV/AIDS, etc.) [13, 20]. Inadequate study or any potential negative effect significantly restrict the use of most antiherpetic drugs in children and women during pregnancy and lactation when the

practical therapist faces the problem of assessing the “risk-benefit” for the patient. In pediatrics, especially in young children, the assortment of antiherpetic drugs is markedly narrow and often limited to acyclovir (over 2 years), inosine pranobex (over one year), as well as Proteflazid®, which can be used from the first days of the child’s life [26]. It should be noted that only Proteflazid® has no direct contraindications for use during pregnancy and lactation [6].

As noted above, when choosing a medication the pharmacokinetic properties of drugs are important as they determine the rate and completeness of delivering an active agent to the target. As a rule, acyclic guanosine analogues, among which is the “golden standard” of antiherpetic drugs – acyclovir, reveal a low bioavailability [18]. Actually most antiherpetic drugs widely used in the clinical practice are acyclovir modifications obtained in order to increase its bioavailability while preserving the activity that is at least not inferior to that of acyclovir [8]. Another important pharmacokinetic characteristic, which determines the efficacy and frequency of prescribing an antiherpetic drug is its elimination half-life [30]. In this aspect, proteflazid exceeds acyclovir and other antiherpetic drugs since its T_{1/2} of about 12 hours determines its therapeutic frequency – twice a day. Antiherpetic drugs used at the pharmaceutical market of Ukraine, except penciclovir, which found the optimal use in external applications, and foscarnet commonly used in resistance of strains to other drugs in the form of injection, are arranged in the following order by the level of bioavailability: proteflazid ≥ famciclovir > valacyclovir > ganciclovir > acyclovir (Table 1).

Another important requirement for antiherpetic drugs is the range of the antiviral action [15]. It is well known that modern antiviral drugs should have a high specificity to the causative agents of herpes infections – herpes simplex virus I and II (HSV I and HSV II), CMV, EBV, human herpes virus type VI A (HHV VI A) and VI B (HHV VI B), etc. Generally, most antiherpetic agents have the activity against different strains of herpes viruses differing on sensitivity to them, and it determines the choice of a drug for the treatment of the disease. It is known that ganciclovir unlike acyclovir, famciclovir, proteflazid and other drugs is more effective against cytomegalovirus infection rather than for the treatment of herpes simplex virus [7, 16, 21]. It has been shown that most EBV-infection symptoms are not associated with the direct cytopathic effect of the virus in the infected tissues, but with the indirect immunopathological response to EBV-infected B-lymphocytes circulating in the blood and being in the cells of the affected organs. That is why the nucleoside analogues (acyclovir, ganciclovir, etc.) and polymerase inhibitors (foscarnet) inhibiting the replication of EBV and reducing the content of the virus in saliva (but not sanitizing it entirely) do not have a clinical effect on severity and duration of symptoms of the EBV-infection, particularly in infectious mononucleosis. In this context, a significant advantage of proteflazid over other antiherpetic drugs is the fact that it has the immunomodulating effect that potentiates the clinical efficacy [6, 26]. The attention should be paid to a serious drawback, which all acyclic nucleosides without exception have: by their mechanism of action they inhibit only actively reproducing (replicating) herpesviruses. Consequently, even the most efficient course of a single use of a chemotherapeutic agent in no way does not prevent a possible recurrence of the same herpes virus infection, or, moreover, a new herpesvirus infection by the related strain or a new type of herpesvirus. This is probably the most serious limitations of the existing chemotherapy of herpesvirus infections

[2]. It should be also noted a significant resistance to acyclovir and other acyclic nucleosides in patients (up to 10%), but concerning proteflazid such data are not available [8]. Thus, in the practice of choosing the optimal antiherpetic drug and taking into account all of the above, the range of products considerably narrows and largely determined by the price of the drug, more specifically by the cost of the course of treatment giving the best result.

The therapy of herpes diseases, depending on their complexity, is long, and usually it is not limited to one drug. Duration of the therapy of herpetic infection is determined approximately and depends on many factors (severity of the disease, the immune system, the properties of the antiherpetic drug, etc.). The clinical experience shows that treatment of herpes infections (in particular, HHV III-V types) is divided into a number of stages [9, 10]. The first stage is the treatment in the acute phase of the disease (or relapse). The antiviral drugs often prescribed topically and orally (parenterally) at the same time are used for the treatment, the course of treatment usually lasts for 5-10 days. The second phase is the treatment in remission. After decline of the main clinical manifestations in order to stimulate completeness of the immune response immunomodulators or herbal adaptogens are used. Drugs of interferon or inducers of their products are also used; symptomatic, health-promoting, physiotherapeutic treatment, and sanitation of sites of infection are performed; the therapy of chronic inflammatory diseases continues. The phase duration is 30- 60 days, depending on the clinical and laboratory parameters of the disease activity [2, 5, 31]. Specific antiviral drugs are the main products at the first stage, and are also used at the second stage of the therapy. Taking into account duration and complexity of herpes treatment the cost of treatment, as well as prophylactic use of antiherpetic drugs, are the main factors. The cost of drugs for treating herpes varies from tens to thousands of US \$ (foscarnet). Considering the low purchasing power of the Ukrainian population the basic price segment of drugs for treating herpes simplex based on the cost of the pack is about \$ 60. Today the range of products for the treatment of infections caused by herpes viruses is limited by the list given in Table 2.

Acyclovir takes one of the central places in the treatment of herpetic infections; it is presented at the Ukrainian market by a significant number of manufacturers (company "Stada Arzneimittel AG", Germany; JSC "Lekhim-Kharkiv", corporation "Arterium", Ukraine, etc.). The average retail price of acyclovir drugs in Ukraine (as for March 2016) ranged from 0.9-1.1 \$ (22-28 UAH) per a pack of domestic producers (Acyclovir, 200 mg, No. 20 produced by PrJSC "Pharmaceutical Firm "Darnitsa", Ukraine) to 5.6 \$ (140 UAH) of foreign manufacturers (Acyclovir, 200 mg, No. 25 produced by "Stada Arzneimittel AG", Germany) [6]. As a rule, duration of the acyclovir treatment for herpes zoster (the acute phase of the disease) is up to seven days. The frequency of administration of the drug – 800 mg 5 times a day – is associated with poor pharmacokinetic properties and often requires the immunomodulatory therapy [18]. The cost of the course of therapy in the acute stage of the disease ranges from \$ 7 to \$ 40 (from 175 to 1000 UAH) depending on the manufacturer of the drug. There are actually proportional expenses with preventive (anti-relapse) use of acyclovir. Valacyclovir presented at the Ukrainian market by a number of manufacturers (JSC "Farmak", Ukraine – Valavir®, JSC "Kyivmedpreparat", Ukraine – Valtrovir, company by "GlaxoSmithKline", UK – ValtreksTM, etc.), has better pharmacokinetic properties, allowing it to apply three times in the dose of 1000 mg for 7 days in the treatment of herpes zoster.

Table 2. The cost of treatment of herpes virus infections

Drug, dose	The average retail price per a pack	Nosology	A course of treatment	Cost of a course
PROTE-FLAZID®, 30 ML	~11\$ / 274 UAH	herpes zoster CMV Infectious mononucleosis	7 drops twice a day for 3 days; 12-15 drops twice a day for the following 2-4 weeks; <i>Maintenance therapy:</i> 10 drops a day for 2-4 months without interruption	33\$ / 830 UAH
Acyclovir, 200 mg, No. 20	from 0,9\$ (22 UAH) to 5,6\$ (140 UAH)	herpes zoster	800 mg 5 times a day for 7 days; <i>Combination Therapy:</i> additionally, interferon alfa-2b 1 million IU intramuscularly + 2 million IU subcutaneously into several points around the area, up to 7 days	from 7\$ (175 UAH) to 40\$ (1000 UAH) additionally from 138 \$ (~3470 UAH)
Valacyclovir, 500 mg No. 10	from 5,2\$ (131 UAH) to 9\$ (230 UAH)	herpes zoster	1000 mg for 7 days; <i>Combination Therapy:</i> additionally, immunostimulant (e.g. polyoxonium 6 mg / day) for 5 days; antioxidants (vitamin B and C groups) intramuscularly five times at intervals every other day	50\$ (~1250 UAH) additionally to 40 \$ (~1000 UAH)
		CMV	2000 mg four times a day for 90 days	520\$ (~13000 UAH)
Ganciclovir, 450 mg, No. 60	from 400\$ (10 000 UAH) to 650\$ (16 500 UAH)	CMV	900 mg (2 tablets a day) for 21 days	from 400\$ (10 000 UAH) to 650\$ (16 500 UAH)
Famciclovir 500 mg, No. 14	107\$ (~2700 UAH)	herpes zoster	1500 mg (3 tablets a day) for 7 days	160\$ (~4050 UAH)

The weighted average cost of the drug varies depending on the manufacturer – from \$ 5.2 (131 UAH) (Valtrovir, 500 mg, No. 10) to \$ 9 (230 UAH) (ValtreksTM, 500 mg, No. 10) [6], so the cost of the course of valacyclovir therapy for herpes zoster can be up to \$ 50 (~ 1250 UAH). Valacyclovir treatment of cytomegalovirus infection is longer (90 days or more) and requires the use of 2000 mg (4 tablets) 4 times a day. The cost of the monthly course increases to \$ 520 (~ 13,000 UAH) and higher [14, 18].

Ganciclovir exhibiting the highest efficiency in different types of herpes infection is the drug of choice for treating cytomegalovirus infection [30]. This drug is in the highest price range, and it is reflected in the cost for the course of treatment (Table 2). At the Ukrainian market the injectable form of the drug dominates (Ganciclovir- Farmeks – LLC “Farmeks Group”, Ukraine; Cymevene, company “F.Hoffmann-La Roche Ltd”, Switzerland), but there is also an oral form (Valcyte 450 mg, No. 60), the price of 1 package reaches \$ 650. For the treatment of cytomegalovirus infection the drug is used in the dose of 900 mg (2 tablets) for 21 days (~ 1 pack). Famciclovir (Famvir, “Novartis Pharmaceutica SA”, Switzerland) reveals significant efficiency, but the price range of the drug and the need for treatment of herpes zoster in the dose of 1500 mg (one tablet three times a day) for 7 days raise the cost of treatment up to more than 100 \$ (~ 4000 UAH) and higher [18].

It is shown that guanosine derivatives exhibit predominantly the antiviral activity, and it requires additional therapies, the use of antioxidants (vitamins E and C) and, if necessary – NSAIDs [3]. The base of modern immunotherapy of herpesvirus infections is drugs of interferon and immunoglobulins. The purpose of such treatment, except for certain indications, corresponds to a high level of evidence. Immunotherapy can not substitute antiviral chemotherapy, but addition of immunotherapeutic drugs can improve the treatment efficacy, reduce the course of treatment and prevent induction of resistance to acyclic guanosine analogues. However, such polypharmacy complicates the treatment of herpes virus infections and often requires involvement of the medical staff, significantly increases the financial costs of treatment [5]. For example, the cost of human recombinant interferon alfa-2a depending on the dosage form, the dose and the manufacturer is in the range from \$ 7 to \$ 95 (from 180 to 2400 USD) per a pack, and human recombinant interferon alfa-2b – 5-34 5 \$ (130 to 870 UAH). Hence, the cost of treatment of herpes zoster with the domestic acyclovir taking into account the daily use of human recombinant alpha-2b interferon (Introferobion PJSC «Pharmstandart- Biolik») according to the scheme of 1 mln of IU intramuscularly, and 2 mln of IU subcutaneously into several points around the affected area with duration up to 7 days increases the treatment costs compared with acyclovir monotherapy by more than 3 times.

Today drugs with the multivector activity (direct antiviral, immunomodulating, interferon-stimulating, antioxidant, apoptosismodulating, etc.) against herpes virus infection are of considerable interest. Proteflazid® possesses these pharmacodynamic properties; its distinctive feature is its natural origin providing tropism to the human body and a high safety profile, therefore, this drug can be prescribed in pregnancy and lactation, as well as for children from birth. In addition, Proteflazid® takes the medium sized niche among other antiherpetic drugs – the weighted average retail price of the drug, as for March 2016, is about \$ 11 (274 UAH). Due to the range of its pharmacodynamic effects Proteflazid® allows to exclude antioxidants, immunomodulators from the antiherpetic therapy scheme, or to adjust their use, and it can significantly reduce the cost per a course of treatment. For example, the cost of treatment of acute herpes using Proteflazid® drug averages \$ 11 (from 288 to 330 UAH) per a month, it is significantly lower than the cost of treatment by other drugs. It should be noted that when using the drug as a therapeutic and preventive (antirelapse) agent for one month only one bottle is enough; it is not only economically feasible for the user, but also increases its compliance for treatment.

Conclusions

1. The accumulated clinical experience with the original Ukrainian drug Proteflazid® (drops) as an effective and safe antiviral drug based on economic expediency are weighty justification in favour of its choice among other antiherpetic drugs.

2. According to the results of the evaluation of the course of treatment of herpes-induced diseases it is expedient to use Proteflazid® (drops) with the therapeutic and preventive purposes since it allows the patient to reduce the cost of treatment and achieve the optimal clinical and economic effect.

References:

1. Дубоссарська З. М., Дубоссарська Ю. А. Герпетична інфекція у жінок: складнощі у вирішенні проблеми. Медичні аспекти здоров'я жінки. – 2007. – №5 (8). – С. 38–40.
2. Ісаков В. А., Архіпова Є. І., Ісаков В. Д. Герпесвірусні інфекції людини. – СПб.: СпецЛіт, 2013. – 670 с.
3. Ісаков В. А., Рибалкін С. Б., Романцов М. Г. Герпесвірусна інфекція (Рекомендації для лікарів). – СПб., 2006. – 96 с.
4. Ісаков В. А., Сільков С. А., Мошетова Л. К., Чернакова Г. М. Сучасна терапія герпесвірусних інфекцій: Посібник для лікарів. – СПб., М., 2004. – 168 с.
5. Казмірчук В. Є., Мальцев Д. В. Рекомендації щодо лікування герпесвірусних інфекцій людини // Український медичний журнал. – 2012. – №5 (91) – IX/X Електронний ресурс. Доступ до файлу: <http://www.umj.com.ua/article/42561/rekomendacii-po-lecheniyu-gerpesvirusnykh-infekcij-cheloveka>
6. Камінський В. В., Шалько М. М., Гриневич О. Й. Протефлазид у вагітних: системний огляд результатів післяреєстраційних спостережень за безпечністю та ефективністю застосування // Здоров'я жінки. – 2014. – №6 (92). – С. 160–164.
7. Кері Ч., Лі Х., Велтє К. Терапевтичний довідник Вашингтонського університету. Пров. з англ. за ред. Д. В. Самойлова та М. А. Осипова, 2-ге російське видання. – М.: «Практика», 2000. – 879 с.
8. Коровіна О. М., Куханова М. К., Кочетков С. Н. Пошук інгібіторів реплікації вірусу герпесу: 30 років після ацикловіру // Biotechnologia Acta. – 2013. – V. 6, №4. – С. 78–85.
9. Кускова Т. К., Белова Е. Г. Сімейство герпесвірусів на етапі. Лікуючий лікар. – 2004. – №5/4. Електронний ресурс. Доступ до файлу: <http://www.lvrach.ru/2004/05/15>
10. Нікулін Л. А., Александрова О. К., Сівак В. В., Боровікова Є. В. Герпетична інфекція (Herpes simplex), 2000. Електронний ресурс. Доступ до файлу: <http://www.infectology.ru/ruk/herpes/index.aspx>
11. Редькін Ю. В., Одокієнко О. Ю. Поліоксидоній у комплексній терапії хворих на рецидивуючий генітальний герпес: клінічна та фармакоекономічна ефективність. Електронний ресурс. Доступ до файлу: <http://www.mediasphera.ru/journals/vestnik/detail/125/1517/>
12. Шестакова І. В., Юшук Н. Д. Сучасні підходи до лікування Епштейна-Барр-вірусної інфекції у дорослих // Лікар. – 2001. – №2 (<http://www.lvrach.ru/2011/02/15435133/>)
13. Akalin E., Sehgal V., Ames S., et al. Cytomegalovirus Disease in High Risk Transplant Recipients Despite Ganciclovir or Valganciclovir Prophylaxis / American Journal of Transplantation. – 2003. – V.3, №6. – P. 731–735.
14. Beeson W. H., Rachel J. D. Valacyclovir Prophylaxis for Herpes Simplex Virus Infection або Infection Recurrence Following Laser Skin Resurfacing Dermatologic Surgery. – 2002. – V. 28, №4. – P. 331–336

15. Brady R. C., Bernstein D. I. Treatment of herpes simplex virus infections // *Antiviral Research.* – 2004. – V. 61, №2. – P. 73–81.
16. Chrisp P., Clissold S. P. Foscarnet. A Review of its Antiviral Activity, Pharmacokinetic Properties and Therapeutic Use in Immunocompromised Patients with Cytomegalovirus // *Retinitis Drugs.* – 1991. – V. 41, №1. – P. 104–129.
17. De S. K., Hart J. C., Breuer J. Herpes simplex virus and varicella zoster virus: recent advances in therapy // *Curr Opin Infect Dis.* – 2015. – V. 28, №6. – P. 589–595.
18. De Clercq E. Antiviral drug in current clinical use // *Journal of Clinical Virology.* – 2004. V. 30, №2. – P. 115–133.
19. Drug Bank database, Drug Bank Version: 3.0 [Электронный ресурс] – доступ до файла – <http://www.drugbank.ca/>
20. Duncan S. R., Grgurich W. F., Iacono A. T., et al. A comparison of ganciclovir and acyclovir to prevent cytomegalovirus after lung transplantation // *American Journal of Respiratory and Critical Care Medicine.* – 1994. – V. 150, №1. – P. 146–52.
21. Faulds D., Heel R., Ganciclovir. A Review of its Antiviral Activity, Pharmacokinetic Properties and Therapeutic Efficacy in Cytomegalovirus Infections // *Drugs.* – 1990. – V. 39, №4. – P. 597–638
22. Acosta E. P., Fletcher C. V. Valacyclovir // *The Annals of Pharmacotherapy.* – 1997. – V. 31, №2. – P. 185–191.
23. Blein C., Gavazzi G., Paccalin M., Baptiste C., Berrut G., Vainchtock A. Burden of herpes zoster: the direct and comorbidity costs of herpes zoster events in hospitalized patients over 50 years in France // *BMC Infect Dis.* – 2015. – V. 19. – №15. – P. 350
24. Bricout H., Haugh M., Olatunde O., Prieto R. G. Herpes zoster-associated mortality in Europe: a systematic review // *BMC Public Health.* – 2015. – V. 15. – P. 466–480.
25. Johnson R. W., Alvarez-Pasquin M.-J., Bijl M. Herpes zoster epidemiology, management, and disease and economic burden in Europe: a multidisciplinary perspective // *Ther Adv Vaccines.* – 2015. – V. 3 (4). – P. 109–120.
26. Kaminsky V. V., Litus O. I., Grynevych O. Y., et al. Proteflazid®: efficacy and safety in urogenital herpes virus infection (systematic review) // *Science and Education Studies.* – 2015. – V. 3, №2(16). – P. 705–727.
27. Korndewal M. J., Mollema L., Tcherniaeva I., van der Klis F. Cytomegalovirus infection in the Netherlands: seroprevalence, risk factors, and implications. // *J Clin. Virol.* – 2015. – V. 63. – P. 53–58.
28. Nilsson J., Cassel T., Lindquist L. Burden of herpes zoster and post-herpetic neuralgia in Sweden // *BMC Infect Dis.* – 2015. – V. 15. – P. 215–221
29. Pinchinat S., Cebrian-Cuenca A., Bricout H., Johnson R. Similar herpes zoster incidence across Europe: results from a systematic literature review // *BMC Infect Dis.* – 2013. – V. 13. – P. 170–183
30. Tan B. H. Cytomegalovirus Treatment // *Curr. Treat. Options. Infect. Dis.* – 2014. – V. 6(3). – P. 256–270
31. White P. J., Garnett G. P. Use of antiviral treatment and prophylaxis is unlikely to have a major impact on the prevalence of herpes simplex virus type 2. // *Sex Transm Infect.* – 1999. – V. 75(1). – P. 49–54.
32. Yawn B., Gilden D. (2013) The global epidemiology of herpes zoster // *Neurology.* – V. 81. – P. 928–930.
33. Whitley R. J., Kimberlin D. W., Roizman B. Herpes Simplex Viruses Clinical Infectious // *Diseases.* – 1998. – V. 26. – P. 541–555.

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7.2. THE PHARMACOECONOMIC BASIS OF USE OF PROTEFLAZID® IN THE TREATMENT OF HUMAN PAPILLOMAVIRUS

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Abstract

The human papillomavirus (HPV) is commonly prevalent. Therapy of HPV is difficult due to the small arsenal of drugs which are effective in the cure of this pathology. Antiviral herbal preparation proteflazid is an effective pharmaceutical for the treatment of HPV. This is due to the wide spectrum of pharmacological activity of proteflazid. Proteflazid's therapeutic activity and capability of correction of auxiliary HPV pharmacotherapy lead to high efficiency of treatment and significant reduction in the cost of treatment of the disease without compromising its effectiveness.

Key words: human papillomavirus infection, proteflazid.

Introduction

Papillomavirus infection is a widespread disease that is usually transmitted sexually [2]. In particular, in the United States, 19.6% – 44.8% of the female population is infected with human papillomavirus (HPV), depending on the age group [1]. According to various researchers, between 1.3% and 72.9% of sexually active men are carriers of HPV. It is known that in most cases, the virus disappears spontaneously; however, in 5–15% of women, HPV causes chronic infection of the cervix, and less frequently, of the vulva or vagina. HPV often affects the anogenital area and can cause both benign and malignant neoplasms [3]. At present, more than 100 genotypes of HPV are known, and at least 15 of them can cause oncological diseases. Chronic infection with oncogenic HPV types has been firmly established as a necessary factor in the development of precancerous conditions, as well as cervical cancer, and tumor lesions of the vulva, vagina, anus, penis, and oropharynx [3]. It has been shown that women with persistent HPV infection in the cervix are 65 times more likely to develop cervical cancer compared to women who do not carry the virus. The risk of disease increases significantly for women over the age of 30 who are infected with high-risk oncogenic strains of HPV (including types 16, 18, 48, 56) [2, 5]. Therapy for this disease is important and relevant, as it preserves the health and life of patients.

HPV belongs to the Papovaviridae family and consists of small DNA viruses, which are characterized by their ability to induce proliferation of the epithelium of the skin and/or mucous membranes [2, 12]. There are about 100 types of HPV that differ in DNA structure, most of which have been molecularly cloned and sequenced. About 30 types of the virus are known to affect the

anogenital area; among them, the following viruses are classified as high-risk oncogenic HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68. The peak of HPV infection is observed in the age range of 18–28 years; however, in older age, due to age-related immunological and hormonal changes, the risk of HPV-related carcinogenesis increases.

Study objective

Pharmacoeconomic analysis of the results of using different medicinal products in the therapy of papillomavirus infection based on available scientific publications.

Study results and their discussion

At present, there are no unified standards for HPV therapy [6, 11]. At the same time, the arsenal of drugs for specific pharmacotherapy of this disease under the current concepts based on evidence-based medicine are limited. Most known drugs that inhibit the replication of herpes simplex virus (acyclovir, ganciclovir, etc.) have proven ineffective in the treatment of anogenital HPV infection [9]. However, it has been shown that some antiviral drugs (lopinavir) have demonstrated activity against HPV in experimental settings [25]. Currently, clinical trials are underway that evaluate the activity and safety of lopinavir in patients suffering from HPV; however, scientifically confirmed data on its efficacy are not yet available [26]. Table 1 presents the main methods of human papillomavirus therapy.

Table 1. Classification of symptomatic/etiotropic methods for combating HPV

Methods	Treatment/preventive care	Therapy type
Destructive	Surgical excision	Symptomatic
Physical	Electrosurgical methods, cryotherapy, laser therapy, radiosurgery, plasma surgery	Symptomatic
Chemical	Nitric acid, trichloroacetic acid, ferezol, solkovagin, solcoderm	Symptomatic
Cytotoxic methods	Podophyllin, podophyllotoxin, 5-fluorouracil	Pro-apoptotic action
Immunological methods	Interferons, isoprinosine, glycopin, immunomax	Immunomodulatory action
Vaccination	Bivalent vaccine, quadrivalent vaccine	Immunomodulatory action
Antiviral therapy	PROTEFLAZID®	Etiotropic (direct antiviral), apoptosis-modulating, immunomodulatory actions

One of the effective methods for combating HPV is vaccination [13]. Currently, two types of HPV vaccines are used in clinical practice, whose mechanism of action is associated with the involvement of cellular immunity as well as the activation of neutralizing antibodies of G class immunoglobulin [8]. They are produced from purified structural L1 proteins that form type-specific HPV empty shells or virus-like particles (VLPs) – HPV-6, HPV-11, HPV-16, HPV-18 – in the quadrivalent vaccine, and HPV-16, HPV-18 – in the bivalent vaccine. The vaccines do not contain live biological products or viral DNA (non-infectious). HPV vaccines are intended for the prevention of the disease, but not for therapy. It should be noted that the vaccine does not protect against all types of HPV, which means it will not prevent all cases of cervical cancer or genital papillomas. Approximately 30% of cervical cancer

cases will not be prevented by vaccination (regular Pap testing is necessary), since the vaccine does not affect other oncogenic strains. The vaccine does not prevent approximately 10% of cases of genital warts either and does not protect against other sexually transmitted infections. It has been shown that vaccination was less effective in young women who were already infected with one of the HPV types covered by the vaccine.

It should also be noted that cervical cancer may develop 15–20 years after HPV infection, which means that the practical experience of using this drug is still insufficient. The retail price of the vaccine averages \$120 per dose. A complete vaccination requires three injections, which raises the total cost of the course to \$360 [7]. In addition, vaccination requires the involvement of appropriately qualified medical personnel.

Table 2. Medicinal products for the treatment of papillomavirus infection

Parameter	PROTE-FLAZID®	Inosine Pranobex	Solco-derm	Podo-phyllotoxin	Podo-phyllin	5-Fluoro-uracil	Imiquimod
Toxicity	Low	Low	–	Toxic	Toxic	Toxic	Moderate
Bioavailability	High, up to 80%	Good >60%	Not absorbed	Low (>0.3%)	Low (>0.3%)	Low (>1%)	Low (>0.9%)
t _{1/2} , hours	5–9	0,9	-	Up to 12 h	Up to 12 h	Up to 10 h	Up to 20 h
Pharmacological action (type of therapy)	Direct anti-viral (HPV), immunostimulatory, antioxidant, apoptosis-modulating	Immunostimulatory action	Destructive action	Pro-apoptotic action	Pro-apoptotic action	Cytostatic action	Immunostimulatory action
Registered in Ukraine	+	+	+	+	-	-	+
Route of administration	Orally / Vaginally	Orally	Topically	Topically	Topically	Topically	Topically
Use in children	From birth	Children over 1 year	Children over 5 years	Prohibited	Prohibited	Prohibited	Not recommended
Pregnancy / lactation	Allowed	Undesirable	Undesirable	Prohibited	Prohibited	Prohibited	Prohibited

Course duration	12–15 drops 2 times daily for 3 months; vaginal tampons/suppositories 2 times a day for 14 days	2 tablets 3 times a day, course of 14–28 days	Single application	4 cycles at 4-day intervals: 2 times a day for 3 days	4 cycles at 4-day intervals: 2 times a day for 3 days	Once at night for 1 week or once a week for 10 weeks	3 times a week for up to 16 weeks
Dosage form	Drops / suppositories	Tablets 500 mg, pack of 50	Solution for external use, 1 ampoule 0.2 ml	Cream, 1 tube, 5 g	Cream, 1 tube, 10 g	Cream, 1 tube, 40 g	Cream, 250 mg sachets, pack of 12
Price per package (UAH)	300/180	180	450	330	786	1836	1600

Among symptomatic drugs with a local cauterizing effect intended for the treatment of HPV, Solcoderm has its specific characteristics (Table 2). Solcoderm is a keratolytic agent, whose active substance is a product of the interaction between organic acids and metal ions with nitric acid. The drug is used in the form of a solution containing nitrates in the amount of 0.02 mg/ml. Solcoderm has a limited local effect on pathologically altered tissues when applied topically. The drug penetrates the treated tissue, subsequently causing mummification of the pathological tissue. As long as the process intensifies, the scab darkens and falls off after a few days. Healing occurs rapidly. A chemical agent that causes a mild destructive effect is 80–90% trichloroacetic acid (TCA). The use of TCA is most effective for small acuminatum or simple papilomas, but is less effective for large or keratinized lesions. The drug often requires repeated application after 1–2 weeks. It should be noted that the use of these agents requires special caution due to the possibility of causing severe pain and the formation of ulcers, and subsequently scars, which often requires the involvement of qualified medical personnel. The price of Solcoderm, manufactured by MEDA Pharmaceuticals Switzerland GmbH, Switzerland, ranges from 394 to 512 UAH. The drug is unstable, so repeated use requires opening of a new ampoule. Successful therapy often requires repeated application of the drug, which significantly increases its cost. Cytostatic and antiviral drugs are also used in the treatment of HPV—podophyllin, podophyllotoxin, 5-fluorouracil (Table 2) [16, 17]. Podophyllin, a resin obtained from the plants *Podophyllum peltatum* and *P. emodi*, is used topically in the form of a 10–25% solution in ethanol or tincture of benzoin. The action of the drug is associated with its effect on the cellular microtubule apparatus, inhibition of mitosis, suppression of nucleic acid transport, which results in the inhibition of DNA synthesis and cell division. The drug is applied 1–2 times per week. Although podophyllin is a drug of choice in the United Kingdom, some researchers describe it as a poorly studied, crudely purified plant extract. In particular, liquid chromatography methods show that approximately 10% of the dry matter of a 20% podophyllin solution consists of two flavonoids—quercetin and kaempferol [28]. Additionally, the use of the drug is limited

by the course duration of up to 5 weeks, as well as by the absence of registration of the drug in Ukraine. Podophyllotoxin is the most therapeutically active fraction of podophyllin. Although podophyllotoxin is postulated as an antiviral agent, it does not exhibit direct antiviral activity against HPV. It prevents the replication of the viruses that cause HPV by acting as a metaphase inhibitor in cells, binding to at least one binding site on tubulin, thus preventing the polymerization of tubulin, which is necessary for the formation of microtubules. Podophyllotoxin is marketed as the drug condyline, in such pharmaceutical forms as solutions (0.25%, 0.3%, and 0.5%) and cream (0.15%, 0.3%, and 0.5%). The drug is applied twice a day for three consecutive days per week, over a 4–5-week course [9, 17]. When using the drug, especially with incorrect application, side effects typical of cytostatics may occur (in up to 15% of patients). Currently, podophyllotoxin is available on the Ukrainian market under the trade name “Wartec” (Stiefel Laboratories Ltd. (Ireland)), with a price per package ranging from 300 to 400 UAH. The cytostatic agent 5-fluorouracil is a pyrimidine antagonist. It has the ability to disrupt the synthesis of both cellular and viral DNA. It is mainly used to treat anogenital HPV in the form of a 5% cream. For intravaginal application, the drug is prescribed once at night for one week, or once a week for 10 weeks [9]. According to various researchers, the effectiveness of the drug is 85–90% [18]. As with other cytostatics, 5-fluorouracil is associated with a wide range of side effects. 5-fluorouracil in the form of a cream is not registered in Ukraine as a medicinal product either, which significantly limits its use for this pathology.

Due to the fact that human papillomavirus persists in epithelial cells and the use of destructive methods does not guarantee the absence of recurrences, interferon therapy appears a promising method in this regard, particularly in combination with other treatment methods. It is possible to perform local, intralesional, and systemic (subcutaneous, intramuscular, or intravenous) interferon administration. It has been established that the use of interferons in patients leads to a reduction in the amount of viral DNA in the lesion sites (according to PCR data), which correlates with clinical improvement or the disappearance of the lesion [9, 14].

Data on the therapeutic effect of interferon inducers have also been obtained. The effectiveness of local application of a low-molecular-weight imidazoquinoline derivative—imiquimod—has been demonstrated that is a cytokine inducer, specifically of LIF (leukemia inhibitory factor) [15]. The greatest therapeutic effect was observed when the drug was used in the form of a 5% cream, applied 3 times per week or nightly until the lesions disappeared (but no longer than 4 months). With daily use, local side effects, such as redness, swelling, and erosion, occurred more frequently. The price of the drug (Aldara, 12 sachets in a package, manufactured by 3M Health Care Limited, United Kingdom) ranges from 1,355 to 1,870 UAH per package, and the cost of a full course of therapy rises to 2,710–3,740 UAH (Table 2). Complete disappearance of condylomas was observed in 13–56% of cases [9].

Effectiveness against HPV has also been shown by the immunomodulator inosine pranobex, which is a complex of inosine and the salt of N,N-dimethylamino-2-propanol and p-acetamidobenzoic acid (Table 2). Inosine pranobex is available in tablet form or syrup form.

According to recent data, the active substance in this complex is likely inosine, while the amino alcohol salt stimulates its penetration through the membrane of lymphocytes and other cells [9, 19]. Inosine pranobex has immunomodulatory effects, associated with enhanced

proliferation of T-lymphocytes induced by mitogens or specific antigens, as well as the differentiation of pre-T-lymphocytes into T-lymphocytes, accompanied by the appearance of specific antigens on their surface, an improved CD4+/CD8+ ratio, and increased production of IL-2 by T-lymphocytes, among other effects, etc. It has been established that the inclusion of inosine pranobex in the complex scheme of treatment contributes to increased effectiveness and helps prevent recurrences of papillomavirus lesions of the cervix [18]. Various treatment schemes of inosine pranobex administration for HPV are used [9, 19, 20, 21]. The recommended dosage is 2 tablets 3 times daily for up to 28 days [9]. Considering the price variation depending on the manufacturer (Novirin, JSC “Kyiv Vitamin Plant”, Ukraine; Groprinosin, Gedeon Richter Plc., Poland; Isoprinosine TEVA, Israel, etc.), which ranges from 200–300 UAH per package of 50 tablets, the cost of a one-month course of therapy may reach 800–1,200 UAH.

Table 3. Treatment schemes for papillomavirus infection and their cost

№	Treatment schemes for papillomavirus infection	Type of therapy	Course cost, UAH
1	Systemic administration of alpha-interferon at a dose of 1.5 to 3 million IU intramuscularly or subcutaneously once in two days for 4 weeks as monotherapy [31]	Immunostimulatory therapy	from 3,000
2	Imiquimod, topical application, 1 sachet 3 times per week for up to 16 weeks [32]	Immunostimulatory therapy	from 4,800
3	Removal of papillomas (solcoderm 1–2 times) combined with intramuscular administration of Immunomax, 200 IU once daily on days 1, 2, 3, 8, 9, and 10 of treatment [33]	Combined (symptomatic + immunostimulatory) therapy	from 1,950–2,400
4	Inosine pranobex, 3 g/day (2 tablets 3 times a day) as an addition to local therapy or surgical intervention for 14–28 days, plus antioxidants [34]	Combined (symptomatic + immunostimulatory) therapy	from 1,750–2,000
5	Inosine pranobex as an addition to surgical removal of papillomas, 5 days per week for 1–2 consecutive weeks per month over 3 months, plus antioxidants [31]	Combined (symptomatic + immunostimulatory) therapy	from 2,800
6	Alpha-interferon: 1 suppository twice a day at 12-hour intervals for 10 days, then 1 suppository twice a day 3 times a week for 2 months, with topical application of alpha-interferon ointment/gel during the first 7 days [36]	Immunostimulatory therapy	from 2,600
7	Combination of destruction (solcoderm), cytotoxic action (podophyllotoxin), and immunomodulation (alpha-interferon 3 million IU intramuscularly 3 times a week, up to 6 months) [34]	Combined (symptomatic + pro-apoptotic + immunostimulatory) therapy	from 10,000
8	Papilloma removal (electrosurgery, laser destruction) with simultaneous intramuscular injections of immunomax, 200 IU once daily on days 1, 2, 3, 8, 9, and 10 of treatment, plus topical 5-fluorouracil [35]	Immunostimulatory + cytostatic therapy	from 3,000

9	Interferon suppositories (viferon) 3 million IU per day, topical viferon gel for 30 days. On day 15 – destruction of papillomas by electrocaugulation [36]	Immunostimulatory therapy	2 600
10	PROTEFLAZID®: week 1 – 7 drops 2 times daily; weeks 2 and 3 – 15 drops 2 times daily; from week 4 – 12 drops 2 times daily; duration – 3 months without interruption. 1 suppository 2 times daily for 14 days. Course duration – 3 months (14 days monthly) [37]	Etiotropic (direct antiviral), pro-apoptotic + immunostimulatory therapy	2 520

At present, various treatment schemes for HPV are used, including both monotherapy and combination therapy (Table 3). Despite the lack of unified standards, complex therapy has shown the highest effectiveness in the treatment of HPV—this includes the use of interferon or its inducers, as well as immune response activators as adjuvant therapy in combination with various destructive methods [30]. It has been shown that immune-based drugs increase treatment effectiveness and help reduce the frequency of relapses. For example, it was found that combining interferon preparations with laser therapy is more effective than laser therapy alone: complete clearance of papillomas was observed in 52–81.5% of patients with the combination therapy versus 19–61% with laser therapy alone. CO₂ laser excision combined with low-dose α -interferon therapy (1–3 million IU per day for 5–7 days, followed by a 3–4 week break, repeated for 3–4 cycles) improves treatment effectiveness and helps reduce the recurrence rate, which is typically observed after laser therapy alone [30]. Clearance of HPV was recorded in 65.6% of patients after combination therapy (isoprinosine + destruction of lesions) for low-grade cervical intraepithelial neoplasia (CIN) [31]. This type of therapy, despite its proven effectiveness, has certain disadvantages, including the need for involvement of medical personnel and a significant increase in treatment cost compared to monotherapy (see Table 3).

The antiviral medicinal product of plant origin with immunotropic properties PROTEFLAZID® has demonstrated significant effectiveness in the treatment of HPV (Table 1). Its pharmacodynamic action is due to flavonoid glycosides that are contained in wild grasses – *Deschampsia caespitosa* L. and *Calamagrostis epigeios* L., as shown in a number of clinical studies [21, 23, 24]. The flavonoids in PROTEFLAZID® inhibit DNA and RNA virus synthesis in infected cells by suppressing the activity of virus-specific RNA and DNA polymerases, thymidine kinase, and reverse transcriptase. The drug also promotes the synthesis of endogenous α - and γ -interferons to a physiologically active level (without inducing the phenomenon of refractoriness), thereby increasing non-specific resistance to viral and bacterial infections. **PROTEFLAZID® is the only medicinal product that has undergone pre-clinical studies on specific antiviral activity against HPV.** In vitro studies on experimental oncogenic models of human papillomavirus have shown that PROTEFLAZID® possesses specific antiviral activity and inhibits the replication of human papillomavirus (HPV) by 2 log ID₅₀, indicating its high antiviral potential [27]. Cytological studies have also established that the active substance suppresses the proliferative and destructive effects of HPV on cells [27]. In clinical settings, the effectiveness of PROTEFLAZID® has been demonstrated in female patients diagnosed with papillomavirus infection and cervical epithelial dysplasia caused by HPV – in more than 80% of cases, HPV replication disappeared in the cells of the multilayered epithelium, and cytological normalization or regression of cervical intraepithelial neoplasia from class CIN-II (moderate dysplasia) to CIN-I (mild dysplasia) was observed [27]. PROTEFLAZID® also exhibits antioxidant activity, which is manifested in the inhibition of free radical processes, and apoptosis-modulating activity, which is realized through its effect on caspase-9 (promoting faster elimination of virus-infected cells).

For the treatment of papillomavirus infection, PROTEFLAZID® is used in the form of vaginal tampons soaked with the drug solution (to prepare the solution, 3.0 ml or 72–75 drops of the medicinal product are diluted in 20.0 ml of saline solution; exposure time – 30–40 minutes, 2 times a day), as well as in the form of suppositories. The course of treatment is 14 days [23].

Oral use of PROTEFLAZID® is also recommended according to the following scheme: 1st week – 7 drops 2 times a day; 2nd–3rd week – 15 drops 2 times a day; from the 4th week – 12 drops 2 times a day, duration is up to 3 months. The combination of oral and vaginal use of PROTEFLAZID®, i.e., combining local and systemic HPV therapy, increases treatment effectiveness. A number of clinical studies have shown that combined use (oral and intravaginal) increases its effectiveness up to 100% [29]. Another advantage of the drug is its high safety profile [22]. Thus, considering that the price of PROTEFLAZID® per package is about 300 UAH (drops) and 180 UAH (suppositories), the cost of a one-month course of therapy, depending on the chosen scheme, may range from 600 to 840 UAH per month [23]. It should be noted that the pharmacodynamic breadth of PROTEFLAZID® allows for adjustments in maintenance therapy of the disease, which can also significantly reduce the cost of treatment.

Conclusions

Pharmacoeconomic analysis indicates that PROTEFLAZID® is the drug of choice for the treatment of papillomavirus infection.

The use of PROTEFLAZID® is justified, as it enables reducing patients' treatment costs and achieving a rational clinical and economic effect.

References:

1. Dunne E., Unger E., Sternberg M. (2007) Prevalence of HPV Infection Among Females in the United States. *JAMA*, vol. 297, no 8, pp. 813–819.
2. Bashmakova M., Savicheva A. Papilomavirusnaya infektsiya (posobie dlya vrachey) [Human papillomavirus infection (manual for physicians)] (electronic journal). Available at: <http://www.lytech.ru/data/file/papiloma.pdf>.
3. Castellsagué X. (2008) Natural history and epidemiology of HPV infection and cervical cancer. *Gynecologic Oncology*, vol. 110, no 3 (2), pp. 84–87.
4. Dunne E., Nielson C., Stone K. (2006) Prevalence of HPV Infection among Men: A Systematic Review of the Literature. *J Infect Dis*, vol. 194, no 8, pp. 1044–1057.
5. Clifford G., Smith J., Aguado T. (2003) Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. *British Journal of Cancer*, vol. 89, pp. 101–105.
6. Budanov P., Voronoy S., Aslanov A. (2004) Printsipy lecheniya papilomavirusnoy infektsii [Principles of treatment of human papillomavirus infection]. *Voprosy ginekologii, akusherstva i perinatologii*, vol. 3, no 4, pp. 70–75.
7. (2009) Vaktsiny protiv virusa papillomyi cheloveka (Rus) [HPV vaccines]. *Weekly Epidemiological Record (WER)*, vol. 84, no 15, pp. 117–132.
8. Stanley M., Lowy D., Frazer I. (2006) Prophylactic HPV vaccines: underlying mechanisms. *Vaccine*, vol. 24, no 3, pp. 106–113.
9. Bogatyireva I. Sovremennyye podhody k lecheniyu papilomavirusnoy infektsii urogenitalnogo trakta [Modern approaches to the treatment of human papillomavirus infection of urogenital tract]. *Lechaschiy vrach* (electronic journal). Available at: <http://www.lvrach.ru/2001/04/4528713>.

10. Derzhavniy reyestr Ikkarskih zasoblv Ukraïny [State register of medicinal products of Ukraine] Available at: <http://www.drlz.kiev.ua>.
11. Centers for Disease Control and Prevention (electronic resource). Available at: <http://www.cdc.gov/std/treatment/2010/genital-warts.htm>.
12. Park I., Introcaso C., Dunne E. (2015) Human Papillomavirus and Genital Warts: A Review of the Evidence for the Centers for Disease Control and Prevention Sexually Transmitted Diseases Treatment Guidelines. *Clin Infect Dis*, vol. 61, no 8, pp. 849–55.
13. Valentino K., Poronsky C. (2015) Human Papillomavirus Infection and Vaccination. *J Pediatr Nurs.*, vol. 213, no 5982–5963.
14. Isakov V., Ermolenko D., Kutueva F. Ispolzovanie tsikloferona v terapii papillomavirusnoy infektsii (Rekomendatsii dlya vrachey) [Cycloferon use in the treatment of human papillomavirus infection (Guidelines for physicians)] (electronic resource). Available at: <http://medi.ru/doc/a210211.htm>.
15. de Witte C., van de Sande A., van Beekhuizen H. (2015) Imiquimod in cervical, vaginal and vulvar intraepithelial neoplasia: a review. *Gynecol Oncol.*, vol. 139, no 11, pp. 77–84.
16. Chong S., Zeng K., Li G., Ren F. (2011) Preparation of podophyllotoxin nanostructured lipid carriers and its effects on immortalized human cervical epithelial cells with HPV infection *in vitro*. *Nan Fang Yi Ke Da Xue Xue Bao*, vol. 31, no 6, pp. 1023–1028.
17. Ramirez-Fort M., Au S., Javed S., Loo D. (2014) Management of cutaneous human papillomavirus infection: pharmacotherapies. *Curr Probl Dermatol*, vol. 45, pp. 175–85.
18. Kolipara R., Ekhlassi E., Downing C. (2015) Advancements in Pharmacotherapy for Noncancerous Manifestations of HPV. *J Clin Med.*, vol. 24, no 4 (5), pp. 832–46.
19. Zabelev A., Dolmatova O., Sivokoneva E. (2005) Rezultaty kolposkopicheskogo skrininga i opyt primeneniya Izoprinozina v lechenii papillomavirusnykh porazheniy sheyki matki [The results of screening and colposcopic experience izoprinozin application in the treatment of papillomavirus cervical lesions]. *FARMATEKA*, no 3, pp. 72–75.
20. Dolgushina V., Ahmatova A., Berenda M. (2009) Effektivnost Izoprinozina v lechenii hronicheskogo tservitsita, assotsiirovannogo s papillomavirusnoy infektsiei [Izoprinozin efficacy in the treatment of chronic cervicitis associated with HPV infection]. *FARMATEKA*, no 14, pp. 73–76.
21. Linask L., Grigoreva E. (2008) Opyit primeneniya Izoprinozina pri zabolevaniyakh sheyki matki na fone papillomavirusnoy infektsii u podrostkov i molodykh zhenschin [Experience with izoprinozin cervical disease from HPV infection among adolescents and young women]. *Russkiy meditsinskiy zhurnal*, vol. 16, no 19, pp. 1–4. «Рецепт», 2016, том 19, №3 371 Фармакоэкономика
22. KamInskiy V., Shalko M., Grynevych O. (2014) Proteflazid u vagItnih: sistemniy oglyad rezultativ pisl-yareEstratslynih sposterezhen za bezpechnIstyu ta effektivnistyu zastosuvannya [Proteflazid in pregnant women: systematic review of observing of post-registration safety and effica]. *Zdorove zhenschiniy*, no 6 (92), pp. 160–164.
23. Kaminskiy V., Shalko M., Grynevych O. (2015) Sravnitel'naya effektivnost i bezopasnost suppozitoriev i kapel Proteflazid® u patsientok s displaziei epiteliya sheyki matki, obuslovennoy papillomavirusnoy infektsiei [Comparative efficacy and safety of suppositories and drops Proteflazid® in patient with epithelial dysplasia of the cervix caused by HPV infection]. *Ukrayinskiy medichniy chasopis*, vol. VII/VIII, no 4 (108), pp. 57–59.
24. Voloshina N., Petrova O., Kuznetsova T. (2007) Monitoring patologii sheyki matki u beremennykh [Monitoring cervical pathology in pregnant women]. *Zdorove zhenschiniy*, no 2 (30), pp. 63–67.
25. Batman G., Oliver A., Zehbe I. (2011) Lopinavir up-regulates expression of the antiviral protein ribonuclease L in human papillomavirus-positive cervical carcinoma cells. *Antiviral Therapy*, vol. 16, pp. 515–525.

26. Lahiri C., Dugan K., Xie X. Oral (2015) Lopinavir Use and Human Papillomavirus Infection in HIVPositive Women. *J Acquir Immune Defic Syndr*, vol. 70 (2), pp. 63–66.
27. Kaminskiy V., Shalko M., Vorobeva L. (2015) Proteflazid® : spetsificheskaya aktivnost v doklinicheskikh issledovaniyakh, effektivnost i bezopasnost primeneniya v klinicheskoy praktike pri zabollevaniyakh, vyizvannykh virusom papillomyi cheloveka (sistematicheskii obzor) [Proteflazid® : efficacy and safety in clinical practice for diseases caused by human papillomavirus (systematic review)]. *Zdorove zhenshiny*, no 3, pp. 128–138.
28. Petersen C., Weismann K. (1995) Quercetin and kaempferol: an argument against the use of podophyllin. *Genitourin Med*, vol. 71, pp. 92–93.
29. Kaminskiy V., Shalko M., Mihaylov V. (2015) Otsenka effektivnosti preparata Proteflazid pri lechenii papillomavirusnoy infektsii: meta-analiz rezultatov mnogoletnih klinicheskikh issledovaniy [Evaluation of efficacy of Proteflazid in the treatment of HPV-infection: meta-analysis of long-term clinical trials results]. *Meditinskii aspekti zdorovya zhenshiny*, no 6 (92), pp. 5–14.
30. Solovov A. (2003) Konservativnaya terapiya porazheniy, vyizvannykh papillomavirusnoy infektsiei [Conservative treatment of lesions caused by HPV infection]. *Lechaschiy vrach*, no 7, pp. 22–26.
31. Gomberg M., Solovov A. (2009) Rekomendatsii patsientam s papillomavirusnoy infektsiei pri otsutstvii ee klinicheskikh proyavleniy [Guidelines for patients with HPV infection in the absence of its clinical manifestations]. *Ginekologiya i dermatovenerologiya*, no 3, pp. 12–18.
32. Grillo-Ardila C., Angel-Müller E., Salazar-Díaz L. (2014) Imiquimod for anogenital warts in non-immunocompromised adults. *Cochrane Database Syst Rev*, Nov 1;11:CD010389 (electronic resource). Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25362229>.
33. Solovov A., Perlamutrov Yu., Ataulhanov R., Pichugin A. (2014) Obosnovanie i opyt primeneniya immunoterapii pri lechenii retsidiviruyushchikh ostrokonechnykh kondilom [Rationale and experience in the application of immunotherapy in the treatment of recurrent condyloma acuminatum]. *Trudnyiye patsient*, no 6, vol. 2, pp. 34–37.
34. Davidson-Parker J., Dinsmore W., Khan M. (1988) Immunotherapy of genital warts with inosine pranobex and conventional treatment: double blind placebo controlled study. *Genitourin Med.*, vol. 64 (6), pp. 383–6. (electronic resource). Available at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1194272>.
35. Fedotov V., Dyudun A. (2008) Immunomaks v terapii hronicheskoy papillomavirusnoy infektsii s soputstvuyushchimi urogenitalnyimi infektsiyami [Immunomax in the treatment of chronic HPV infection associated with urogenital infections]. *Dermatovenerologiya, kosmetologiya, seksopatologiya*, no 1–2 (11), pp. 225–228.
36. Molochkov A. (2009) Immunoterapiya genitalnoy papillomavirusnoy infektsii [Immunotherapy of genital human papillomavirus infection]. *Lechaschiy vrach*, no 5 (electronic resource). Available at: <http://www.lvrach.ru/2009/05/9208767/>.
37. Instruktsiya dlya medichnogo zastosuvannya Ilkarskogo zasobu Proteflazid® [Manual for medical use drug Proteflazid®] (electronic journal). Available at: <http://www.drlz.com.ua/>



Appendices

APPROVED
Order of the Ministry
of Health of Ukraine
No. 2143 dated 21.09.2020
Registration certificate
No. UA/4220/01/01

CHANGES ARE MADE
Order of the Ministry
of Health of Ukraine
No. 2131 dated 25.11.2022

INSTRUCTION
for medical use of medicinal product
PROTEFLAZID®
(PROTEFLAZIDUM®)

Composition.

1 ml of drops contains 1 ml of liquid extract Proteflazid (content of flavonoids not less than 0,32 mg/ml equivalent to rutin, content of carbonic acids not less than 0.30 mg/ml equivalent to apple acid) from Tufted hair grass (*Herba Deschampsia caespitosa* L.) and bush grass (*Herba Calamagrostis epigeios* L.) (1:1).

Extraction solvent: Ethanol 96%.

Dosage form. Drops.

Main physical and chemical properties: dark greenish liquid with a specific odor. .

Pharmacotherapeutic group.

Direct-acting antiviral drugs Code ATX J05A X.

Pharmacological properties.

Pharmacodynamics

Flavonoids which compose the medicinal product are capable of inhibiting replication of DNA- and RNA- viruses both in vitro and in vivo. Inhibitory activity of the medicinal product towards herpes virus, hepatitis, human papillomaviruses, HIV-infection, flu and ARVI has been revealed and proved in pre-clinical and clinical studies.

It has been established that the mechanism of direct antiviral action includes the inhibition of virus specific enzymes – DNA- and RNA-polymerases, thymidine kinase, reverse transcriptase, neuraminidase.

Proteflazid inhibits the activity of the 3CL-protease of the SARS-CoV-2 coronavirus, which has been confirmed by molecular docking and by using an assay kit containing 3CL-protease tagged with MBP (maltose-binding protein of the SARS-CoV-2 coronavirus).

Using the Renilla luciferase dual-reporter gene assay (reproducing replication of the seasonal coronavirus CoV-229E), it was shown that the medicinal product blocks it.

In preclinical in vitro studies on monkey (Vero E6) and human (A549/ACE2) cell cultures, the antiviral activity of the medicinal product against the pandemic human coronavirus SARS-CoV-2 was demonstrated – with significant inhibition of virus replication.

The medicinal product has immunotropic properties. The medicinal product protects mucosae, normalizing the local immunity parameters (lactoferrin, secretory immunoglobulin A, lysozyme and C₃ component of the complement).

It has been established that the medicinal product is the inductor of synthesis of endogenous α - and γ -interferons to physiologically active level, increasing organism's non-specific resistance to viral and bacterial infections.

Clinical studies showed that the daily administration of the medicinal product according to the age-specific dosage and treatment scheme does not cause immunotoxicity and does not cause immune system's refractoriness (hyporeactivity), and inhibition of the synthesis of α - and γ - interferons that enables patients to use the medicinal product for a long time, if necessary.

The medicinal product possesses antioxidant activity, inhibits free radical processes. Thus, it prevents accumulation of lipid peroxidation products, enhances anti-oxidant status of cells, decreases intoxication, facilitates recovery after infectious disease and adaptation to adverse environmental conditions.

The medicinal product is an apoptosis modulator, which intensifies the activity of apoptosis-inducing factors, specifically: activates caspase 9, resulting in faster elimination of cells affected by virus and primary prevention of chronic diseases on the background of latent viral infections.

The medicinal product prevents disease recurrence and prolongs remission period.

Pharmacokinetics.

Active ingredients of the medicinal product are quickly absorbed from gastro-intestinal tract into blood, reaching maximum concentrations within 20 minutes after administration (study in vivo). Taking into account the present dynamics, half-life excretion from blood plasma takes 2,3 hours.

Bioavailability by oral administration makes 80%. Excretion from the body is slow. The level of accumulation of active substances in blood cells is much higher in comparison with blood plasma. Corresponding concentrations of active substances in blood provide prolongation of action of the medicinal product in the body and accumulation in organs and tissues as a result of their release from blood cells. Such pharmacokinetic dynamics of accumulation and release of active substances by blood cells determine requirement to take the medicinal product twice a day to reach the effective concentrations.

Clinical characteristics.

Indications.

Treatment and prophylaxis of recurrences caused by:

- herpes simplex virus type 1 and 2;
- herpes zoster viruses and chicken pox (type 3);
- human herpesvirus type 4 (Epstein-Barr virus), acute and chronic active forms;
- human herpesvirus type 5 (cytomegalovirus).

Treatment and prophylaxis of flu and other ARVI (in particular, flu pandemic strains).

In a complex treatment of:

- hepatitis B and C;
- viral, bacterial, fungal infections and their associations (chlamydia, mycoplasma, ureaplasma, etc.);
- HIV-infection and AIDs.

Etiotropic therapy of light and medium forms of cervical dysplasia (CIN1 and CIN2) caused by human papillomavirus (HPV) infection, including oncogenic strains.

As a part of a complex therapy of other forms of diseases caused by human papillomavirus infection, including oncogenic strains.

Contraindications.

Hypersensitivity to the ingredients of the medicinal product. Gastroduodenal ulcer.

Interaction with other medicinal products and other forms of interactions.

During clinical use it was established that it is possible and justified to combine Proteflazid® with antibiotics and anti-fungal drugs for treatment of viro-bacterial and viro-fungal diseases. No negative manifestations associated with interaction with other drugs have been found.

Administration details.

During the treatment of viral hepatitis in 10-15 % of patients with expressed cytolytic syndrome in 2-4 weeks after the beginning of treatment, the increase of activity of aminotransferases, less frequently – of bilirubin level, which lasts for 2-4 weeks and does not require withdrawal of the drug, can be observed.

Patients with gastro-intestinal disorders and chronic gastroduodenitis in case of exacerbation of gastroduodenitis or esophageal reflux should take the drug 1,5-2 hours after meal.

Transient increase of the body temperature to 38 °C does not require the withdrawal of the drug. In case of increase of the body temperature it is necessary to contact your doctor to exclude other possible causes of that phenomenon.

It is necessary to decrease the concentration of the medicinal product in the solution for application if it causes itching or dryness while being applied topically.

To avoid urinogenital reinfection, simultaneous treatment of a sexual partner is recommended.

Use during pregnancy or breastfeeding.

Pre-clinical studies did not reveal any embryotoxic, teratogenic, fetotoxic, mutagenic and oncogenic effects. No special studies related to such influence of the medicinal product on a human fetus have been conducted, but clinical experience of use of the drug in I-III trimesters of pregnancy and lactation period did not reveal any negative influence. A doctor should decide whether it is justified to use the medicinal product during pregnancy or breastfeeding.

Effects on ability to drive and use machines.

No negative influence of the medicinal product on potentially hazardous activities that require special attention and quick reaction has been revealed.

Posology and method of administration.

Shake the vial before use.

The medicinal product should be dosed with a dropper. The necessary quantity of the medicinal product should be dropped into water (in the volume of 1-2 tablespoon) and should be taken 10-15 minutes before meal.

Age-based dosing of Proteflazid® (drops)

Age (years)	Dosing (drops) and dosing frequency per day
From birth to 1 year	1 drop once a day
From 1 to 2 years	1 drop twice a day
From 2 to 4 years	2 drops twice a day
From 4 to 6 years	4 drops twice a day
From 6 to 9 years	9 drops twice a day
From 9 to 12 years	10 drops twice a day
From 12 years and adults	12-15 drops twice a day

Duration of Proteflazid® use depends on indications and course of disease.

For treatment and prevention of recurrence of herpetic gingivostomatitis, pharyngotonsillitis, chicken pox; for complex treatment of viral, bacterial and mycotic infections and their associations; to avoid viral and bacterial infections which arise in patients with insufficient function of immune system it is recommended to take medicinal product during 1 month.

For treatment of eczema herpeticum and herpetic vesicular dermatitis (together with local application of the solution); herpetic meningitis and encephalitis, herpetic eye lesion; genital herpes; treatment of herpes zoster, acute and chronic active forms of Epstein-Barr virus; cytomegalovirus; human papillomavirus (together with local application of the solution) it is recommended to take the medicinal product during 3 months without a break.

In case of recurrent infections, the treatment course should be taken 1-2 times per year according to the recommendation of a doctor.

Duration of treatment in children is the same as in adults. Doses should be prescribed depending on patient's age, and course of a disease. Treatment should be carried out under doctor's control.

In a complex therapy of viral hepatitis B and C, HIV-infection and AIDS, it is recommended to take the medicinal product during 6-12 months without a break.

Treatment of hepatitis in HIV-infected and AIDS patients should be carried out before or after antiretroviral therapy.

For treatment of flu and ARVI, the medicinal product should be taken during 5-14 days depending on the course of a disease. For prophylaxis, the medicinal product should be taken during 2-4 weeks in the dose, which makes one-half of the curative dose. During epidemic, the duration of the medicinal product use may be extended up to 6 weeks.

Local administration simultaneously with oral intake of the medicinal product:

For treatment of infections of herpes simplex of skin and mucosae, acute forms of herpes zoster and skin papillomatosis, the solution of the medicinal product should be applied on the affected area 3-5 times a day. The exposure period is 10-15 minutes. To prepare solution it is necessary to dissolve 1,5 ml (36-38 drops) in 10 ml of physiological solution of sodium chloride.

Local application should be continued until signs of skin or mucosal lesion disappear, but not less than for 10 days.

In case of primary or recurrent genital herpes, human papillomavirus and viro-bacterial infections of female genital organs, vaginal tampons with the medicinal product solution are applied. To prepare solution it is necessary to dissolve 3,0 ml (72-75 drops) in 20 ml of physiological solution of sodium chloride. The exposure period of vaginal tampons application is 30-40 minutes; manipulation should be made twice a day.

Duration of local use of vaginal tampons in case of genital herpes is 10 days, in case of human papillomavirus, viral and bacterial infections of female genital organs – 14 days.

Children.

Proteflazid® can be used by children from birth.

Overdose.

No cases of overdose are known, however development of adverse reactions, especially in gastrointestinal tract is possible. Symptomatic treatment is recommended.

In case of overdose of Proteflazid®, it is necessary to immediately consult a doctor.

Adverse reactions.

Allergic reactions: in patients with increased sensation, hypersensitivity reactions are possible. Rare allergic reactions, including erythematic rash and itching are possible.

Gastrointestinal disorders: very rare cases of gastrointestinal disorders – pain in epigastric region, nausea, vomiting, diarrhea can be observed. In patients with chronic gastroduodenitis exacerbation of gastroduodenitis, gastroesophageal reflux (reflux esophagitis) are possible.

General disorders: very rare cases of headache, general weakness, transient increase of body temperature to 38 °C on the 3-10th day of treatment can be observed.

Laboratory parameters: during treatment of viral hepatitis, in 10-15% of patients with pronounced cytolytic syndrome, increase of aminotransferases activity (less frequently – of bilirubin level) can be observed.

Local reactions: in case of local application, burning, itching, dryness may be observed. Contact your doctor if any adverse reactions occur.

Shelf-life.

3 years.

Do not use after the end of the shelf-life specified on the package.

Storage conditions.

Store in the original package at the temperature less than 25 °C. Do not freeze! Formation of a gel-like structure which is destroyed by shaking is possible. Keep out of reach of children.

Package.

Light-protective glass vials of 10 ml, 30 ml or 50 ml, capped with tamper-evident caps with dropper plugs or tamper-evident and child-resistant closure with dropper plugs in carton package.

Category of prescription.

By prescription.

Manufactured by.

Scientific and Manufacturing Company “Ecopharm” LTD.

Location of the manufacturer and address of the activity.

116 Shevchenko St., Ulashanivka village, Shepetivskyi district, Khmelnytskyi region, 30070, Ukraine.

Applicant.

Scientific and Manufacturing Company “Ecopharm” LTD.

Address of the applicant.

136-B Naberezhno-Korchuvatska St., Kyiv, 03045, Ukraine

APPROVED
Order of the Ministry
of Health of Ukraine
No. 2220 dated 30.09.2020
Registration certificate
No. UA/4220/02/01

CHANGES ARE MADE
Order of the Ministry of
Health of Ukraine
No. 2271 dated 15.12.2022

INSTRUCTION
for medical use of medicinal product

PROTEFLAZID®
(PROTEFLAZIDUM®)

Composition:

active ingredient: 1 suppository contains Proteflazid flavonoids derived from the mixture of herbals (1:1) of Tufted hair grass (Herba *Deschampsia caespitosa* L.) and Bush grass (Herba *Calamagrostis epigeios* L.) not less than 1,8 mg;

excipients: butylhydroxyanisole (E 320), polyethylenglycol-400, polyethylenglycol-1500, polyethylenglycol-4000, until the mass 3 g is received.

Dosage form. Suppositories.

Main physical and chemical properties: brown-green torpedo-shape suppositories.

Pharmacotherapeutic group.

Direct-acting antiviral drugs Code ATC J05A X.

Other gynecological drugs. Code ATC G02C X.

Pharmacological properties.

Pharmacodynamics.

The active ingredient of the medicinal product (flavonoids) inhibits synthesis of DNA- and RNA-viruses in the infected cells through the inhibition of activity of virus-specific enzymes: RNA-, DNA-polymerases, thymidine kinase and reverse transcriptase; possesses immunotropic properties. It has been established that the active ingredient facilitates synthesis of endogenous α - and γ -interferons to physiologically active level (without refractoriness) increasing local non-specific resistance to viral and bacterial infections.

Clinical studies have shown that PROTEFLAZID® (suppositories) restores the protective function of mucous membrane of the vagina and cervix uteri normalizing the parameters of local immunity (sIgA; lysozyme and complement component C₃).

Studies in experimental models of oncogenic human papilloma viruses in vitro proved that the active ingredient of the medicinal product has a specific antiviral activity and inhibits reproduction of human papillomavirus on 2 Ig ID50.

Cytological studies have established that the active substance inhibits proliferative and destructive impact of Human papillomavirus over cells.

During the treatment of epithelial dysplasia of cervix caused by papillomavirus infection, the normalization of a cytological image or transition from cervical intraepithelial neoplasia class CIN-II (moderate dysplasia) to class CIN-I (mild dysplasia) has been registered.

In the case of genital herpes, the medicinal product prevents formation of new elements of rash, decreases the likelihood of dissemination and visceral complications and accelerates regenerative processes.

In the case of vaginosis, vaginitis and inflammatory diseases of cervix uteri, the medicinal product facilitates the local immune reconstitution and faster and more effective elimination of the pathogen.

The medicinal product facilitates the elimination of dysbiotic disorders of genital tract microflora, reconstructs normal vaginal biotope, accelerates processes of reconstruction of epithelium of endocervix; prevents recurrences of the diseases.

The medicinal product possesses antioxidant activity, which inhibits free-radical processes, thus preventing accumulation of lipid peroxidation products, enhances anti-oxidant status of cells.

The medicinal product is an apoptosis modulator, which intensifies the effect of apoptosis-inducing factors, activating caspase 9, resulting in faster elimination of virus infected cells and primary prevention of chronic diseases on the background of latent viral infections.

Pharmacokinetics.

The active ingredient does not penetrate into general blood circulation and has no systemic exposure, when applied topically. The studies proved that by vaginal use the therapeutic concentration of the medicinal product is reached locally.

Clinical characteristics.

Indications.

Treatment of diseases of female genital organs caused by:

- herpes simplex virus types I and II, cytomegalovirus and Epstein-Barr virus;
- human papillomavirus (HPV), including oncogenic strains. As a part of complex treatment of female genital diseases caused by:
- pathogens of inflammatory diseases of mixed etiology (viruses, bacteria, pathogenic fungi, chlamydia, mycoplasma, ureaplasma).

Contraindications.

Individual hypersensitivity to the medicinal product ingredients.

Special safety precautions.

Suppositories should not be used orally.

The medicinal product does not require any special precautions.

Interaction with other medicinal products and other forms of interactions.

PROTEFLAZID® (suppositories) can be combined with other antiviral drugs and antibiotics for the treatment of viro-bacterial and viro-fungal diseases of pelvic organs. No negative manifestations associated with interaction with other drugs have been found.

In the event of any adverse reaction caused by combined use of the medicinal product, please contact your doctor.

Peculiarities of use.

It is recommended to avoid sex intercourse during the treatment by the suppositories.

Etiopathogenetical therapy of diseases specified in section “Indications”, except for local therapy with PROTEFLAZID® (suppositories), should be supplemented with oral use of PROTEFLAZID® (drops) according to the schemes and at the dosage specified in the respective instruction.

To reach a desirable therapeutic effect in the treatment of genital diseases caused by pathogens of viral, bacterial, fungal infections and their associations (chlamydia, mycoplasma, ureaplasma)

simultaneous treatment of both sexual partners is necessary. In this case, PROTEFLAZID® (drops) should be used for treatment of a partner according to the schemes and the dosage specified in the respective instruction.

The medicinal product contains butylhydroxyanisole (E 320), and therefore may cause local skin reactions (for example, contact dermatitis) or irritation of mucous membranes.

Use during pregnancy or breastfeeding.

Pre-clinical studies of the active ingredient of PROTEFLAZID® did not reveal any toxicological, teratogenic, mutagenic, embryotoxic, fetotoxic and oncogenic effects. Clinical experience of the use of PROTEFLAZID® (drops) in I-III trimesters of pregnancy and lactation period did not reveal any negative impact.

It is necessary to follow the drug prescription rules during pregnancy or breastfeeding by evaluating the risk-benefit ratio. The medicinal product should be administered only according to prescription and under doctor's supervision.

Effects on ability to drive and use machines

No negative influence of the medicinal product on potentially hazardous activities that require special attention and quick reaction has been revealed.

Posology and method of administration.

Suppositories must be used vaginally.

The suppositories should be used after hygienic procedures. It is necessary to remove the protective plastic packaging before the use of suppositories. Suppository should be inserted deeply into vagina. It is recommended to stay in a lying position for at least 3 hours and to avoid sexual intercourse minimum for 8 hours, after inserting the suppository. It is recommended to start treatment immediately after menstruation. It is necessary to interrupt the treatment during menstruation period.

To treat genital diseases caused by herpes virus types I and II, use 1 suppository once a day for 7-10 days and more until the symptoms disappear.

To treat recurrent herpes infection, including the presence of cytomegalovirus infection or Epstein-Barr infection, use 1 suppository once a day for 10 days. Course of treatment is 3 months (10 days per month).

In case of papillomavirus infection and/or herpes infection in combination with bacterial and fungal infections use 1 suppository twice a day for 14 days. Course of treatment lasts 3 months (14 days per month).

Children.

The use of PROTEFLAZID® suppositories in children has not been studied. Therefore, the medicinal product should not be administered to children.

Overdose.

No cases of overdose are known. In case of overdose, it is necessary to immediately consult a doctor concerning further treatment.

Adverse reactions.

The use of the medicinal product can cause mild local itching or mucous membrane irritation that usually disappear spontaneously and do not require discontinuation of therapy.

Hypersensitivity and allergic reactions are possible.

In case of allergic reactions or any other adverse reactions, stop using suppositories and contact your doctor concerning further therapeutic tactics.

Shelf-life.

2 years.

Storage conditions.

Store in the original package out of reach of children at temperature not above 25°C.

Do not freeze!

Keep out of reach of children.

Package.

5 suppositories in a blister, 1, 2 or 3 blisters in a pack.

Category of prescription.

By prescription.

Manufactured by:

Scientific and Manufacturing Company “Ecopharm” LTD, Ukraine

Location of the manufacturer and address of the activity:

116 Shevchenka St., Ulashanivka village, Shepetivskyi district, Khmelnytskyi region, 30070, Ukraine.

Applicant:

Scientific and Manufacturing Company “Ecopharm” LTD.

Address of the applicant:

136-Б Naberezhno-Korchuvatska St., Kyiv, 03045, Ukraine.

APPROVED
Order of the Ministry
of Health of Ukraine
No. 1032 dated 25.05.2021
Registration certificate
No. UA/5510/01/01

CHANGES ARE MADE
Order of the Ministry
of Health of Ukraine
No. 2131 dated 25.11.2022

INSTRUCTION
for medical use of medicinal product

FLAVOVIR®

Composition:

1 ml of syrup contains:

0,02 ml of liquid extract of Proteflazid obtained from the mixture of herbals (1:1) tufted hair grass (Herba *Deschampsia caespitosa* L.) and bush grass (Herba *Calamagrostis epigeios* L.) (extraction solvent – ethanol 96 %), which is the equivalent of not less than 0,0035 mg of flavonoids equivalent to rutin;

Excipients: propylene glycol, ethanol 96 %, sorbite (E 420), methylparaben (E 218), propylparaben (E 216), sodium sulphite (E 221), purified water.

Dosage form. Syrup.

Main physical and chemical properties: transparent sweet yellow-greenish liquid with a faint specific smell.

Pharmacotherapeutic group.

Direct-acting antiviral drugs Code ATX J05A X.

Immune stimulants. Code ATX L03A X.

Pharmacological properties.

Pharmacodynamics.

Flavonoids which compose the drug are capable of inhibiting replication of DNA- and RNA-viruses both in vitro and in vivo. Inhibitory activity of the drug towards influenza virus, acute viral respiratory infections (AVRI) and herpes viruses has been revealed in pre-clinical and clinical studies.

It has been established that the mechanism of direct antiviral action includes the inhibition of synthesis of virus specific enzymes – DNA- and RNA-polymerases, thymidine kinase, reverse transcriptase, 3CL protease, neuraminidase and induction of synthesis of endogenous interferon.

The flavonoids in the syrup inhibit the activity of the 3CL protease of the SARS-CoV-2 coronavirus, as confirmed by the method of molecular docking and through the use of a test kit containing 3CL protease labeled with MBP (maltose-binding protein of the SARS-CoV-2 coronavirus). Using the dual-luciferase reporter gene assay with Renilla luciferase (which affects the replication of the seasonal coronavirus CoV-229E), its inhibition was demonstrated.

In preclinical in vitro studies on Vero E6 and A549/ACE2 cell cultures, antiviral activity against the pandemic human coronavirus SARS-CoV-2 was demonstrated, with significant inhibition of viral replication.

The medicinal product protects mucosa of upper respiratory tracts, normalizing the local immunity parameters (lactoferrin, sIgA and lysozyme).

During the studies it has been established that the medicinal product normalizes synthesis of endogenous α - and γ -interferons to a physically active level, increasing organism's non-specific resistance to viral and bacterial infections.

Clinical studies showed that the daily administration of the drug according to the age-specific dosage and scheme does not induce immune system's refractoriness: inhibition of the synthesis of α - and γ -interferons is not revealed. This characteristic of Flavovir® syrup maintains the interferon level sufficient for an adequate immune response of an organism against infectious agent. In its turn, this allows for using the drug for a long time, if necessary.

The medicinal product possesses antioxidant activity and inhibits free radical processes. Thus, it prevents accumulation of lipid peroxidation products enhancing anti-oxidant status of cells, decreases intoxication, facilitates recovery after infectious disease and adaptation to adverse environmental conditions.

The medicinal product is an apoptosis modulator: it intensifies the activity of apoptosis-inducing factors, activating caspase 9 that encourages faster elimination of cells affected by virus and primary prevention of chronic diseases on the background of latent viral infections.

Pharmacokinetics.

Active ingredients of the medicinal product are quickly absorbed from gastrointestinal tract into bloodstream, reaching maximum concentrations within 20 minutes after administration (study in vivo). Taking into account existing dynamics, the plasma elimination half-life is approximately 2.3 hours. Bioavailability at oral administration makes 80%. Elimination is slow. The level of accumulation of active ingredients in blood cells is much higher in comparison to blood plasma. Corresponding concentrations of active ingredients prolong the action of the medicinal product in a human body and accumulation in organs and tissues as a result of their release from blood cells. Such pharmacokinetic dynamics of accumulation and release of active ingredients from blood cells determines requirement to take the medicinal product twice a day to reach the effective concentrations.

Clinical characteristics.

Indications.

- Etiotropic treatment and prevention of ARVI;
- etiotropic treatment and prevention of influenza, in particular influenza caused by viruses of pandemic strains.

Contraindications.

Hypersensitivity to the ingredients of the medicinal product. Stomach ulcer or duodenal ulcer in acute stage. Autoimmune diseases.

Interaction with other drugs and other forms of interactions.

During clinical use it was established that Flavovir® syrup can be combined with antibiotics and antifungal drugs for treatment of viro-bacterial and viro-fungal diseases. No negative manifestations associated with interaction with other drugs have been found.

Administration details.

Patients with chronic gastroduodenitis in case of exacerbation of gastroduodenitis with esophageal reflux symptoms should take syrup 1.5-2 hours after meal.

This medicinal product contains 3.85% vol. ethanol (alcohol), i.e., from 15.4 mg per dose (for children from birth to 1 year old) up to 277.2 mg per dose (for children aged 12 years and older,

and adults), which is equivalent to 0.4 ml of beer / 0.2 ml of wine (for children from birth to 1 year old) up to 6.9 ml of beer / 2.9 ml of wine (for children aged 12 and older, and adults) per dose.

Harmful for patients with alcoholism. Use with caution in pregnant and breastfeeding women, children, and patients with liver disease or epilepsy.

If a patient has an established intolerance to certain sugars, it is necessary to consult a doctor before taking this medicinal product, as it contains sorbitol.

Methylparaben and propylparaben may cause allergic reactions (possibly delayed).

Sodium sulfite may rarely cause hypersensitivity reactions and bronchospasm.

3Use during pregnancy or breastfeeding.

Pre-clinical studies did not reveal any teratogenic, mutagenic, embryotoxic, fetotoxic and oncogenic effects. Clinical experience of use of the medicinal product in I-III trimesters of pregnancy and lactation period did not reveal any negative influence. Nevertheless, it is necessary to observe the rules of prescription of drugs during pregnancy or breastfeeding, estimating benefit-risk profile and consult a doctor.

Effects on ability to drive and use machines.

No negative influence of the medicinal product on potentially hazardous activities that require special attention and quick reaction has been revealed.

Posology and method of administration.

Shake the vial with syrup before use.

The syrup should be dosed using dosing container and taken orally 20-30 minutes before meal. To increase therapeutic effect in case of respiratory viruses it is recommended to keep the syrup for 20-30 seconds in the mouth, gargling before swallowing.

Dosage and duration of treatment depend on type of disease and age of a patient.

Dosages of the medicinal product

Patient's age	Dosing
From birth to 1 year	0.5 ml twice a day
From 1 to 2 years	1 ml twice a day
From 2 to 4 years	3 ml twice a day
From 4 to 6 years	4 ml twice a day
From 6 to 9 years	5 ml twice a day
From 9 to 12 years	6 ml twice a day
From 12 years and adults	9 ml twice a day

The syrup should be used during 5 days for treatment of influenza and ARVI (without complications). To increase the effectiveness of the therapy, use syrup at the first manifestations of a disease or after a contact with patients. The treatment may be prolonged to 2 weeks, depending on the course of the disease.

For prevention of influenza and ARVI half of the curative dose of the syrup should be used during 1-4 weeks.

The term of using the syrup for prevention care can be extended to 6 weeks during epidemic with the spread of pandemic strains.

To normalize the immune system in case of bacterial complications of influenza and other ARVI, the syrup should be used for 4 weeks and longer.

Children.

Flavovir® can be used for children since birth.

Overdose.

No cases of overdose are known. In case of the overdose of the syrup, please contact your doctor.

Adverse reactions.

Allergic reactions: hypersensitivity reactions can be observed in patients with increased sensation. Allergic reactions, including rash, itching, skin edema, urticaria and skin hyperemia can be observed.

Gastrointestinal disorders: pain in epigastric region, nausea, vomiting, diarrhea can be observed (if any of these symptoms are present, take the syrup 1,5-2 hours after meal). Exacerbation of gastroduodenitis and gastroesophageal reflux (reflux esophagitis) are possible in patients with chronic gastroduodenitis.

General disorders: transient increase in body temperature up to 38 °C on the 3-10th day of treatment and headache can be observed.

If you experience any adverse reaction, please contact your doctor.

Shelf-life. 2 years.

Do not use after the end of the shelf-life specified on the package.

Storage conditions.

Store in the original package at the temperature less than 25 °C. Do not freeze.

After the first opening of the vial keep it closed in the original package not longer than for 30 days.

Package.

Dark glass or plastic vials of 30 ml, 50 ml or 60 ml capped with plastic tamper-evident caps or plastic tamper-evident caps and child-resistant closure in carton package. A dosage container is placed into carton package.

Category of prescription.

Without prescription.

Manufactured by:

Scientific and Manufacturing Company “Ecopharm” LTD.

Location of the manufacturer and address of the activity:

116 Shevchenko St., Ulashanivka village, Shepetivskyi district, Khmelnytskyi region, 30070, Ukraine.

Applicant:

Scientific and Manufacturing Company “Ecopharm” LTD.

Address of the applicant:

136-B Naberezhno-Korchuvatska St., Kyiv, 03045, Ukraine.

NATIONAL CONSENSUS ON THE MANAGEMENT OF PATIENTS WITH CERVICAL INTRAEPITHELIAL NEOPLASIA CAUSED BY HUMAN PAPILLOMAVIRUS INFECTION

Work group:

V. M. Zaporozhan – Moderator, President of the Association of Obstetricians and Gynecologists of Ukraine, Doctor of Medical Sciences, Professor, Academician of the National Academy of Medical Sciences of Ukraine, Vice-President of the National Academy of Medical Sciences of Ukraine, Rector of Odesa National Medical University;

V. V. Kaminskyi – moderator, Doctor of Medical Sciences, Professor, Corresponding Member of the National Academy of Medical Sciences of Ukraine, Head of the Department of Obstetrics, Gynecology and Reproduction at Shupyk National Medical Academy of Postgraduate Education,

Chief Freelance Specialist of the Ministry of Health of Ukraine in the specialty “Obstetrics and Gynecology”, Director of Kyiv City Center for Reproductive and Perinatal Medicine;

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Pathological processes of the cervix (Cx) acquire particular significance both in the clinical practice of obstetrician-gynecologists and in the broader context of public health. Their frequency is very high and shows no signs of decreasing. Timely identification and adequate treatment of both background processes and precancerous cervical diseases make it possible to prevent the development of cervical cancer (CC). That is why the issue of early diagnosis and treatment of cervical pathological conditions is of particular importance for patients' reproductive health, especially given the asymptomatic nature of these conditions may cause its progression. This is particularly relevant in today's emancipated society, where men often yield to women in family support and child care.

Numerous studies by morphologists, oncologists, gynecologists, and immunologists show that DNA-containing viruses, such as human papillomaviruses (HPVs), are risk factors for the development of cervical cancer that are considered an etiological factor for this pathology.

The non-integrated form of infection is characterized by the production of intact viral particles, and at this stage, papillomavirus infection (PVI) is often asymptomatic and highly pathogenic.

It is now proven that viral infection can remain latent for several years, and its presence significantly increases the risk of developing cervical cancer—on average, by 300 times in the case of persistence of high-risk carcinogenic strains [4, 5, 7].

PVI is diagnosed in 10% of patients with background cervical conditions (diseases). Therefore, the issue of PVI deserves special attention, as the presence of a viral factor places patients in a high-risk group for developing cervical cancer. According to numerous literature sources, HPV infection is linked with cancer in 50–84% of cases.

HPV infection can be classified as a disease that affects the country's demographic indicators, since HPV is a sexually transmitted disease. During pregnancy, due to changes in immunity, latent HPV becomes active and accelerates tumor transformation of the cervical epithelium. Infection is also possible during pregnancy and childbirth.

Most often, young women and men aged 20–30 affected by the infection, see an oncologist at a late stage, as the transition to a malignant form occurs slowly and is practically imperceptible to the patient.

Therefore, this document presents key information about the symptoms of HPV infection and provides recommendations on the management strategy for patients, with the aim of preventing cancer incidence among women diagnosed with this infection.

Definition

Human papillomavirus infection is a condition caused by human papillomavirus (HPV), which can replicate in the human body. Like other papillomaviruses, it has the ability to infect keratinocytes of the skin or mucous membranes. Most known types of HPV cause asymptomatic disease progression, but some lead to frequent development of papillomas, keratoses, warts, and also to the development of malignant neoplasms.

Epidemiology

Cervical diseases occupy a leading place in the structure of reproductive system pathology and are detected in 15–20% of women of childbearing age and in 5–9% of postmenopausal women.

Even cases of benign background processes account for 35–80% of all visits for gynecological care, according to various data.

Every year, around half a million women worldwide who suffer from cervical cancer (CC) are registered, and up to 270,000 of them die within the first year after diagnosis.

Cervical cancer ranks second among gynecological oncological diseases in the countries of the European Union (EU). Each year, approximately 33,000 new cases of cervical cancer and up to 15,000 deaths from this disease are reported in the EU[1]. The incidence of cervical cancer in Ukraine significantly exceeds global statistical data (11.04 vs. 7.94 per 100,000 women), and the mortality rate is nearly twice the global average (4.9 vs. 3.2 per 100,000 women).

As for the prevalence of HPV infection, it is currently the most widespread one worldwide with a global prevalence rate of 11.7%. According to epidemiological studies, at least 95% of squamous cell cervical cancer cases contain HPV DNA. Globally, about 630 million people are infected with HPV [8, 10, 12]. It is important to note that the peak of HPV infection occurs in the 20–25 age group that is during the period of women's reproductive and sexual activity. The most frequently detected high-oncogenic risk HPV genotypes are: HPV-16 (47%), HPV-18 (29%), HPV-52 (9%), HPV-31 (8%), and HPV-58 (7%) [2].

This infection is considered one of the main causes of precancerous conditions and cervical cancer. Epidemiological studies that established the link between HPV and cervical cancer were published in the 1990s [2, 6, 7, 9]. According to WHO data (1986), the development of squamous cell and glandular cervical cancer is closely associated with promiscuous sexual behavior, frequent changes of sexual partners, and exposure to sexually transmitted infectious agents.

Etiology

It has been proven that the main cause of cervical cancer development (72–90% of cases) is persistent HPV infection. The causative agent of HPV infection is a DNA-containing virus that belongs to the Alpha papillomavirus genus of the Papillomaviridae family, which affects the basal membranes of mucous membranes.

HPV cells are distinguished by the genetic sequence of the outer capsid protein L1. More than 30 strains of HPV infect the genital tract. Today, HPV types are classified into high, potentially high, and low oncogenic risk groups. HPV types of low risk include: 6, 11, 34, 35, 40, 42, 43, 44, 54, 55, 61, 62, 70, 71, and 74. Potentially high-risk types include: 26, 53, and 66.

High-risk HPV types include: 16, 18, 31, 33, 45, 51, 52, 58, 59, and 39 [1, 3, 4].

One of the causes of cervical pathology is induced abortion, which leads to trauma, neuroendocrine disorders, and infections that indirectly affect the immature (underdeveloped) cervical epithelium. Inflammatory diseases of the female reproductive organs can also lead to cervical pathology – this is a pressing medical issue that significantly affects the health of millions of women of reproductive age. Important contributing factors to HPV-related diseases include early onset of sexual activity, number of sexual partners, prolonged use of oral contraceptives, infertility, obesity, and smoking.

Women who smoke belong to the high-risk group for the development of precancerous conditions and cervical cancer. Researchers G. I. Rzepka and co-authors (1993), as well as S. E. Waggoner and co-authors (1994), showed that nicotine accumulates in cervical mucus in proportion to its concentration in the blood. Although its typical concentrations do not cause a pathological effect, but a selective mitogenic effect of nicotine on ectocervical cells has been observed, especially when these cells are affected by HPV.

There is evidence that every third sexually active woman exhibits various clinical manifestations of HPV infection. Most often (in 95% of cases), HPV is localized in the transformation zone of the cervix, where cervical epithelial neoplasia occurs. HPV influences on pathological proliferation of the columnar epithelium. High-risk oncogenic HPV types are the main etiopathogenetic cause of cervical neoplasias. HPV integrates into the genome of basal epithelial cells and disrupts their genetic stability, which leads to the development of cervical cancer.

The processes, promoting the regeneration of cervical areas and the formation and growth of reserve epithelial cells depend on many factors. Directly or indirectly, the condition of the cervix is influenced by such factors as age, inflammatory processes and their nature, infections, hormonal and immune status of the body. Factors that increase the risk of developing cervical pathology include hormonal and immune disorders, menstrual cycle disorders, abortions, inflammatory processes in the reproductive organs, and others, especially in the presence of HPV infection.

Diagnostics

The problem of diagnosing and treating precancerous and cancerous cervical diseases associated with HPV has become particularly relevant in recent years due to a sharp increase in the incidence of cervical cancer, the high oncogenic potential of the virus, the ineffectiveness of traditional therapy, and the lack of specific antiviral treatment.

A particular feature of HPV infection is the fact that, due to the virus's epitheliophilic properties, it is not detected in the blood, and the immune system's production of antibodies is observed in far from all infected individuals, owing to the body's inability to provide long-term and reliable immunity due to the very low level of antibodies [1, 3–5]. As a result, HPV DNA persists in the cervical epithelium for a long time, leading to atypical transformation of epithelial cells. Consequently, up to 88% of patients with cervical epithelial neoplasia show cytological signs of HPV, and in 80.2% of cases, infection of the cells is located precisely in the transformation zone [2, 3].

The philosophy of cervical screening is based on the “three pillars”: cytology with HPV testing, colposcopy, and histology. The Pap test is the most widely used in various countries of the world. It is the most successful and most cost-effective test in the history of medicine that can help to prevent all types of cervical cancer if performed correctly.

Screening for cervical pathology is a method of preventing the development of oncological process by detecting and treating pathological changes in the cervical epithelium of women that, if left untreated, may develop into invasive cervical cancer. If these changes are identified in time and the woman is treated, educated about prevention methods, and regularly monitored by a doctor, cervical cancer can be prevented [15]. Unfortunately, the potential of cytological screening is still underutilized, as it remains opportunistic that is performed upon a patient's request. Systematic screening can prevent up to 80% of cervical cancer cases, as has been demonstrated in many countries of the European Union.

Instrumental Method

Clinical manifestations of cervical pathology can be clearly detected through vaginal examination using simple and extended colposcopy.

Colposcopy allows the clinician to examine the genital tract (tissues) with magnification ranging from 8 to 40 times. The use of a 3% acetic acid test enables the identification of atypical acetowhite epithelium (AWE). The presence of AWE is an important colposcopic marker, utilized in the most recent International colposcopic terminologies (Rio de Janeiro, 2011). Certain epithelial changes in damaged areas of the epithelium, i.e. subclinical lesions (abnormalities) that present well-defined white patches, granular or smooth surfaces, and vascular changes, help identify the location for further cytological examination and biopsy.

Colposcopy is an important method for the in-depth diagnosis of pathological changes in the cervical epithelium. In many countries, colposcopy is not used for cervical cancer (CC) screening. It also requires specialized training and the physician's practical experience in order to avoid diagnostic and treatment errors related to cervical pathology.

In visual diagnostic methods for cervical pathology, the Schiller's test is also used; it involves applying an aqueous Lugol's solution to the cervix.

Cervical epithelial cells that contain glycogen stain brown. In the presence of cervical diseases, the amount of glycogen decreases, and accordingly, absorption is reduced. Such areas are considered Lugol-negative zones. If they are identified, further examinations are required, such as colposcopy and HPV testing, as indicated. During extended colposcopy, the condition of the cervical and vaginal epithelial covering is examined, along with areas showing pathological changes.

Signs of HPV infection in the colposcopic image include the following changes:

- acetowhite epithelium;
- condylomas acuminatum and flat condylomas;
- glands with rims; in the areas where glandular ducts are located, rims of acetowhite (dysplastic metaplastic) epithelium appear, which may indicate persistent HPV infection. In such cases, a targeted biopsy must be performed for histological examination to exclude cancer presence;
- atypical vessels and atypical transformation zones;
- mosaic pattern;
- punctation (according to modern terminology, 2011);
- keratosis (leukoplakia);

• Iodine-negative epithelial areas. From any pathologically altered area, a sample must be taken for cytological examination, and subsequently, if indicated, for histological examination.

International Classification of Colposcopic Terms, adopted at the VII World Congress on Cervical Pathology and Colposcopy (Rome, 1990):

I. Normal colposcopic findings:

1. Multilayered squamous epithelium.
2. Columnar epithelium.
3. Normal transformation zone.

II. Abnormal colposcopic findings:

A. Within the transformation zone:

I. Acetowhite epithelium:

- a) flat;
- b) micropapillary or microfolded.
2. Punctuation.
3. Mosaic pattern.
4. Keratosis (leukoplakia).
5. Iodine-negative epithelium.
6. Atypical vessels.

B. Outside the transformation zone (ectocervix, vagina):

1. Acetowhite epithelium:

- a) flat;
- b) micropapillary or microfolded.
2. Punctuation.
3. Mosaic pattern.
4. Leukoplakia.
5. Iodine-negative epithelium.
6. Atypical vessels.

III. Suspicion of invasive cancer on colposcopy.

IV. Unsatisfactory colposcopy:

1. The border of the multilayered squamous epithelium is not visualized.
2. Pronounced inflammation or atrophy.
3. The cervix is not visualized.

V. Mixed findings:

1. Acetowhite micropapillary surface.
2. Exophytic condyloma.
3. Inflammation.
4. Atrophy.
5. Ulcer.
6. Other.

Colposcopic classification of pathological processes of the cervix (Kokhanevych Ye. V., Hanina K. V., et al., 1967, 1997).

I. Benign (background) pathological processes:

1. Ectopy of columnar epithelium:
 - a) dishormonal;

- b) post-traumatic.
- 2. Benign transformation zone (zone of benign metaplasia):
 - a) incomplete transformation zone;
 - b) complete transformation zone.
- 3. Inflammatory processes of the cervix:
 - a) exocervicitis;
 - b) endocervicitis.
- 4. True erosion.
- 5. Benign polyp-like formations.
- 6. Cervical endometriosis.

II. Precancerous conditions of the cervix:

- 1. Simple leukoplakia
- 2. Dysplasia fields:
 - a) stratified squamous epithelium;
 - b) metaplastic columnar epithelium.
- 3. Papillary dysplasia zone:
 - a) multilayered squamous epithelium;
 - b) metaplastic columnar epithelium.
- 4. Pre-tumor transformation zone.
- 5. Condylomas.
- 6. Precancerous polyps.

III. Preclinical cervical cancer:

- 1. Proliferative leukoplakia.
- 2. Fields of atypical epithelium.
- 3. Papillary zone of atypical epithelium.
- 4. Zone of atypical transformation.
- 5. Zone of atypical vascularization.

IV. Clinically manifested cancer:

- 1. Exophytic form.
- 2. Endophytic form.
- 3. Mixed form.

International Classification of Colposcopic Terms (IFCPC, Rio de Janeiro, 2011).

I. General principles:

1. Adequate/inadequate colposcopic image (with specified reason: objective assessment of the cervix is complicated due to inflammation, bleeding, scar changes, etc.).

2. Border between multilayered squamous and columnar epithelium (completely visualized, partially visualized, not visualized).

3. TZ (Transformation Zone) Type I, II, III.

II. Normal colposcopic findings:

- 1. Multilayered squamous epithelium (mature, atrophic).
- 2. Columnar epithelium (ectopy).
- 3. Metaplastic epithelium (nabothian cysts, open glands – crypts).
- 4. Deciduous (during pregnancy).

III. Abnormal colposcopic findings:

- 1. General principles:

a) localization of the lesion (within or outside the transformation zone, according to the clock-face position);

b) size of the lesion (expressed as a percentage in relation to the endocervix).

2. Grade I (mild lesion):

a) thin acetowhite epithelium with irregular, indistinct borders:

- delicate mosaic pattern;

- delicate punctuation.

3. grade II (pronounced lesion):

a) dense acetowhite epithelium with well-defined borders;

b) rapid whitening;

c) dense acetowhite rim around open glands (crypts):

- coarse mosaic pattern;

- coarse punctuation;

- within the lesion – contours of a denser acetowhite area;

- evidence of nodularity (ridges).

4. Non-specific signs:

a) leukoplakia;

b) erosion,

c) areas stained with Lugol's solution (Schiller's test):

- iodine-positive;

- iodine-negative.

IV. Suspicion of invasion:

1. Atypical vessels. Additional signs: "fragile" vessels, uneven surface, exophytic lesion, areas of necrosis and ulceration.

V. Other colposcopic findings:

1. Congenital transformation zone.

2. Stenosis. Condylomas.

3. Congenital anomalies.

4. Polyps.

4. Consequences of previous treatment.

5. Inflammation.

7. Endometriosis.

Laboratory diagnostics

The first stage of cervical pathology screening is cytological examination.

This procedure can be performed by a general practitioner or even a nurse after special training and preparation. This is because the quality of material collection for cytology determines both the diagnostic accuracy and a woman's health. Material for cytological examination must be collected from the surface of the cervix, the transformation zone, and the cervical canal.

This allows the cytologist to assess the cervical epithelium and detect the presence of intraepithelial lesions of varying degrees of malignancy.

The father of cervical cytology is considered to be Dr. George Papanicolaou, who in 1928 published the article "A New Diagnosis of Cancer." In the article he described a test that made it possible to detect cervical cancer at early stages. In 1943, together with Herbert Traut, he published the monograph "Diagnosis of Uterine Cancer by the Vaginal Smear." The book described a method of cytological examination using a staining technique that to this day is

associated with his name—Papanicolaou staining. This became the foundation for the development of screening programs in many countries in the following decade. The implementation of these programs made it possible to reduce the incidence and mortality from cervical cancer by 50–80% (70) [12].

Screening for Cervical Pathology is not a test for cancer. It detects possible abnormalities or changes in cells that may transform into cancer if not prevented. Most changes detected during cervical cancer screening are successfully treated. The quality of cytological material collection and the accuracy of laboratory conclusions based on smear microscopy are important components of the screening test.

However, the widespread use of traditional cytology has certain weaknesses, namely:

- a high percentage of low-quality smears requiring repeated sample collection;
- difficulties in accurately assessing the cellular material (insufficient or excessive number of cells, overlapping, mechanical cell damage, improper fixation, presence of blood cell and inflammation elements).

All this may lead to the possibility of delayed detection of cells with precancerous changes due to a dense background or incorrect evaluation of the nature of the changes caused by overlapping cells and other influencing factors. The diagnostic sensitivity of the cytological method on average accounts for 60%, which means that in 40% of cases the results do not correspond to the degree of existing changes (i.e., they become false negative).

In particular, in 1996, the method of liquid-based cytology (LBC) was introduced in the United States. The difference between this method and traditional cytology lies in the fact that the collected cervical epithelial cells are placed in a special liquid medium and sent to the laboratory, where the smear is prepared using specialized equipment and then stained.

Liquid-based cytology helps eliminate some of the limitations of traditional cytology. This, in turn, contributes to an increase in the diagnostic sensitivity of cytological screening.

Distinctive Features of the Liquid-Based Cytology Method:

- the entire sample collected from the cervix is preserved;
- wet fixation in a preservative liquid enhances the clarity of cellular structures and prevents the formation of artifacts during air drying;
- the ability to obtain a thin, representative monolayer sample allows for timely detection of individual cells with precancerous changes and provides justification for further examination;
- the possibility of preparing additional samples (up to five) if the first one turns out to be unsatisfactory. The method allows storing samples for an extended period: 4 weeks in the container and 6 weeks under refrigeration;
- the number of unsatisfactory samples unsuitable for evaluation is reduced;
- sensitivity to mild forms of neoplasia is increased;
- the specificity of the overall cytological method is improved;
- the sample can be used for additional testing: detection of HPV infection, identification of proliferation markers p16/Ki67, and other markers.

Clinicians should take into account that the quality of cytological examination, in terms of ensuring the effectiveness of secondary prevention (regardless of which smear preparation method is used), depends on:

- a) strict adherence to the rules for preparing the patient for cytological screening;
- b) the specifics of obtaining a complete and adequate sample [10].

The effectiveness of the traditional Pap test depends on the patient's preparation, quality of sample collection by the gynecologist, proper application onto the glass slide, adequate and timely wet fixation, correct staining of the samples, and accurate interpretation of cellular changes by the cytologist.

The most effective staining method in the world is recognized to be Papanicolaou staining, as polychromatic dyes allow for clear visualization of both cytoplasmic and nuclear structures.

One of the factors that ensures the effectiveness of cytological examination within the framework of cervical screening is the referral form for analysis, which should include patient's passport information, clinical diagnosis, date of last menstruation or duration of menopause, and any treatment received (such as hormone replacement therapy or use of oral contraceptives). All these data allow for an adequate assessment of the nature of changes in the cervical epithelium and an objective cytological conclusion. In the laboratory, the cytologist first evaluates the adequacy of the material. According to the criteria for a smear suitable for interpretation, it must contain 8,000–12,000 squamous epithelial cells for traditional cytology or 5,000 cells for liquid-based cytology. Whereas, there must be at least 10 cells of columnar and metaplastic epithelium. After evaluating the sample, the cytologist makes a cytological conclusion based on the nature of the cellular changes, following accepted classification systems [12].

Today, several classification systems exist:

- for cytological examination using the Papanicolaou method (CIN classification), the Bethesda System (TBS) is used. This classification was adopted in 1988 in Bethesda, Maryland, USA. It was supplemented and partially revised in 2001, 2006, and 2012. The most recent update was made in 2014. This classification is recommended worldwide as the optimal system for issuing cytological reports. It defines a two-tier system for analyzing squamous intraepithelial lesions (LSIL and HSIL), which reflects the biology of changes occurring in HPV infection: productive (episomal), corresponding to LSIL, and integrated, corresponding to HSIL.

- for histological samples, the WHO classification and the CIN classification are used.

At present, the main international guidelines in cytological screening recommend providing cytological conclusions according to the Bethesda System (2014), which includes the following categories of changes:

- NILM – negative for intraepithelial lesion or malignancy.

For evaluating **squamous epithelium** of the cervix:

- LSIL – low-grade squamous intraepithelial lesion;
- HSIL – high-grade squamous intraepithelial lesion;
- ASCUS – atypical squamous cells of undetermined significance;
- ASCH – atypical squamous cells cannot exclude HSIL;
- Squamous cell carcinoma – invasive squamous cell carcinoma.

For evaluating **columnar epithelium** of the cervix:

- AGC favor neoplastic – atypical glandular cells, favor neoplastic;
- AGCUS – atypical glandular cells of undetermined significance;
- AIS – endocervical adenocarcinoma in situ;
- adenocarcinoma.

One of the features of this classification is the identification of the ASCUS/ASCH categories for squamous epithelium and AGCUS/AGC favor neoplastic for glandular epithelium, which makes it possible to timely suspect the presence of precancerous conditions and justify the need for more in-depth examination of the woman. Issuing a cytological report according to the Bethesda System (TBS) helps standardize the format of cytological conclusions worldwide, making it possible to exchange experience in managing various types of pathology.

The distinction of ASCUS/ASCH categories for squamous epithelium and AGCUS/AGC favor neoplastic allows for an increase in the diagnostic sensitivity of cytological screening.

Today, cytological examination remains the main method of cervical cancer screening in many countries worldwide.

The classification unites the work of a cytologist and gynecologist. It helps convey what the cytologist observed and helps the gynecologist determine the further management strategy for the patient. Using the classification, the physician must identify the clinically significant information for decision-making. This is because information about smear types is far from everything the cytologist intends to transfer to the gynecologist. Classification should comply with the latest advancements concerning neoplasia.

It is clear that the terminology used in the Bethesda classification differs from the traditional types commonly used: NILM, LSIL, HSIL, ASCUS, ASCH, AGC.

It should be noted that when using this classification, it is not recommended to apply the term LSIL to describe glandular epithelium. It is important to mention that “mild to moderate dysplasia” of columnar epithelium is not included in the Bethesda classification, only severe dysplasia (AGC) is included in this classification.

Also it is necessary to emphasize neoplastic cellular changes, particularly squamous metaplasia, which is often misinterpreted. If staining is performed incorrectly, such metaplasia may be interpreted as keratosis.

Hyperkeratosis, dyskeratosis, metaplasia, and atrophic changes are all variants of normal, although these changes do not exclude HPV infection.

It is important to consider that cellular pathology is divided into two subtypes:

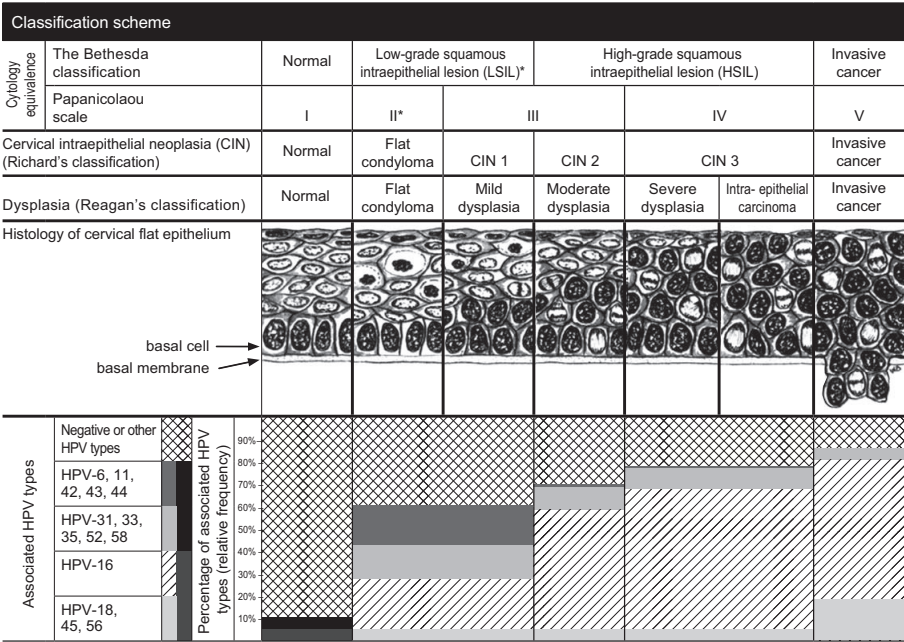
pathology of the (squamous) multilayered squamous epithelium, and pathology of the glandular epithelium. Due to the differences in glandular epithelial pathology, there is an absolute mismatch with the classification by traditional types. Currently, an increase in the number of cases of glandular epithelium pathology and endocervical cancer has been noted [13, 15].

The incomplete transformation zone and the crypts of the glandular epithelium in the exo- and endocervix are the most vulnerable areas for HPV penetration and for the development of cervical glandular intraepithelial neoplasia (CGIN) – a condition in which no pathology is detected on the exocervix, but glandular neoplasia and its most severe form – adenocarcinoma – are identified in the endocervix during colposcopy and the Pap test [13].

Screening programs in the USA and some EU countries include HPV testing for women over 30 years old, which is explained by the proven role of HPV in the development of cervical cancer (see figure). The inclusion of a type-specific DNA test allows the identification, along with cytological changes, of a group of women at risk for cervical intraepithelial neoplasia, even when cytology appears normal. In the part of HPV-infected individuals, the virus undergoes self-elimination, but in 10–15% (or 1 in every 10–30 HPV-infected women), cytological atypia is detected. However, the ability to eliminate HPV is predominantly observed

in young women under 25 years of age. After 30, persistence of HPV is more frequently (and increasingly) observed. It should be taken into account that smoking and immunosuppression double the risk of progression of cervical intraepithelial neoplasia to CIN III in HPV-positive women.

That is why HPV testing has become the foundation of the latest WHO recommendations for screening, diagnosis, and treatment of cervical cancer lesions.



* LSIL also includes atypical squamous cells of undetermined significance (ASCUS)

Figure. Correlation of various classifications. From condyloma to cancer

The cytological interpretation under the Bethesda System (TBS) also includes pathogenic microorganisms (Trichomonas, Candida-like fungi, shift in flora characteristic of bacterial vaginosis, bacteria resembling actinomycetes, and cells typical of herpes simplex virus and cytomegalovirus). Special attention must be given to viruses. The physician must understand exactly what is being referred to: herpes simplex and cytomegalovirus, or HPV.

What should a gynecologist know upon detecting HPV infection? This infection is characterized by a latent form, which does not manifest cytologically. The virus manifests cytologically in its productive phase by a specific marker – koilocytosis.

Based on the analysis of large cytological databases, it has been established that even with ASCUS, cancer in situ (CIS) may be diagnosed in 2% of cases. With LSIL, CIS may be diagnosed in 10% of cases, and invasive cancer – 0.2%.

According to the recommendations of American colleagues, in such cases colposcopy and biopsy are not necessary, but at the same time, there is no need to return to routine screening of the patient. Instead, co-testing should be repeated in 6 and 12 months. In the case where atypia or

an HPV positive result is detected, standard examination is carried out using colposcopy, biopsy, and cervical curettage. If no abnormalities are found in the cytological examination, the patient is returned to routine screening.

The latest WHO recommendations propose the screen and treat strategy. Screening begins with an HPV test. If a woman is virus-positive, visual examination is then performed.

If an acetowhite lesion or iodine-negative area is observed, cryodestruction is recommended without performing biopsy or colposcopy. This is WHO expert recommendation. Cryodestruction is preferred compared to conization, since conization has a higher risk of bleeding and preterm birth. However, experts also report a lower recurrence rate of the disease when conization is performed.

But is this recommendation suitable for our context? To implement the screen and treat system, it is necessary to take a step backward. The answer to this question can be found in the same guidelines, which state that cytology, colposcopy, biopsy followed by treatment (i.e., the approach we are accustomed to) – are acceptable methods and have the right to be used if they are already well established. It is important to clearly understand that this respected organization issues guidelines not only for Europe, but also for African and other countries with low levels of social, economic, and medical development. The proposed approach is relevant only for those regions of Ukraine where cytological screening cannot be performed. The key value of this document lies in consideration of the economic, social, and medical characteristics of each country when choosing a strategy for cervical screening.

There are interesting recommendations of the American Society for Colposcopy and Cervical Pathology, which, like the WHO document, were issued in 2013 and represent a revised 2006 consensus guidelines. During this period, data from 1.5 million women with dysplasia from a large center in North Carolina were analyzed and tracked depending on cytological materials and biopsy findings.

The American screening system recommends to begin screening from the age of 21. From 21 to 29 years, liquid-based cytology (LBC) is performed every three years. LBC allows for the evaluation not only of the cell morphology, but also for the analysis of HPV DNA. In the age group from 30 to 65 years, HPV DNA typing is added to cytology – this is referred to in the literature as “co-testing” – cytology and HPV testing performed simultaneously. This type of examination is done once every five years. Also, it is possible to perform only LBC every three years.

Therefore, if a patient has normal cytology but is HPV positive, she must come under medical supervision and undergo periodic follow-up examinations after six months and one year.

Only if HPV presence is confirmed repeatedly, further examination is indicated, since presence of HPV indicates its persistence.

In practice, according to the Bethesda classification, the diagnosis “Atypical Squamous Cells of Undetermined Significance” (ASCUS) is established relatively rarely, that is cells that do not exclude the possibility of high-grade lesions. However, precisely within this category of patients the probability of severe or moderate dysplasia is quite high and it requires colposcopy and biopsy. In cases where colposcopy does not detect a lesion, co-testing is performed, followed by colposcopy performed twice at 6-month intervals, after prior anti-inflammatory treatment.

During colposcopy, a targeted biopsy and histological examination may be performed to evaluate the morphological changes in cervical tissue. This procedure is considered delayed in time. To collect material, a biopsy must be taken from the cervical area showing pathological changes.

To make the procedure diagnostically significant, it is necessary to compare two tissue samples. One sample is taken from the area of the cervix with the most pronounced changes, and the other – from an unchanged cervix area. To obtain material for examination, the procedure is performed under colposcopic guidance. The obtained sample must include both the epithelium and stroma.

WHO Histological Classification (2003)

I. Squamous cell tumors and their precursors:

1. Squamous cell carcinoma, unspecified:

- a) keratinizing;
- b) non-keratinizing;
- c) basaloid;
- d) verrucous;
- e) warty;
- f) papillary;
- g) lymphoepithelioma-like;
- h) papillary squamotransitional carcinoma.

2. Microinvasive squamous cell carcinoma.

3. Squamous intraepithelial neoplasia.

4. Squamous cell carcinoma in situ.

5. Benign squamous lesions:

- a) condyloma acuminatum;
- b) squamous papilloma;
- c) fibroepithelial polyp.

II. Glandular tumors and their precursors:

1. Adenocarcinoma:

- a) mucinous adenocarcinoma:
 - endocervical adenocarcinoma;
 - intestinal adenocarcinoma;
 - signet-ring cell adenocarcinoma;
 - malignant adenoma;
 - villoglandular adenocarcinoma.

b) endometrioid adenocarcinoma;

c) mesonephric adenocarcinoma;

d) clear cell adenocarcinoma;

e) serous adenocarcinoma.

2. Microinvasive adenocarcinoma.

3. Adenocarcinoma in situ.

4. Glandular epithelial dysplasia.

III. Other epithelial tumors:

1. Adenosquamous carcinoma:

- a) glassy cell variant.
- 2. Adenoid cystic carcinoma.
- 3. Adenoid basal carcinoma.

4. Neuroendocrine tumors:

- a) carcinoid;
- b) atypical carcinoid;

- c) small cell carcinoma;
 - d) large cell neuroendocrine carcinoma.
5. Undifferentiated carcinoma.

The histological examination method in HPV diagnostics has a high prognostic value (over 90%), but low sensitivity (only 50%). This explains the necessity of supplementing histological examination with a method of viral infection detection using PCR. This molecular biological method makes it possible to detect signs of infection long before the first clinical manifestations or cellular changes appear, and can very accurately determine the specific type of virus [14]. Another important step in determining the integration of the viral genome, especially in repeated HPV-positive results, is the detection of cellular biomarkers p16 and Ki67. The presence of both markers confirms the proliferation stage – the irreversible stage of integration of viral DNA into the human genome, when self-elimination becomes almost impossible.

In modern conditions, this method has become widely used. It belongs to amplification-based methods for the detection of papillomavirus DNA. When conducting a complex examination that includes histological analysis and the use of HPV DNA detection methods, it is possible to significantly reduce the incidence and mortality from cervical cancer. Thanks to such complex diagnostic approach, it is possible to control the concentration of the virus in the body, based on which conclusions can be drawn regarding the course and prognosis of the disease.

The treatment tactics for severe forms of lesions is well known and well developed. The only point that requires special attention concerns patients who have atypical glandular cells or adenocarcinoma in situ noted in their examination reports. Such women, as well as all women over the age of 35, and patients with obesity, oligomenorrhea, or abnormal uterine bleeding, should undergo, in addition to cervical curettage under colposcopic guidance, an endometrial curettage.

In the work of an oncogynecologist there is one important aspect: the examination results must correlate with each other, that is cytology should confirm colposcopy, and colposcopy should confirm histology. In cases where such correlation is absent, a diagnostic conization biopsy is indicated. An exception is young women under 24 years of age, since their risk of developing invasive cancer is low.

Treatment

Today, HPV treatment is carried out using various methods; however, eliminating the virus from the body is quite difficult. At the current stage, the treatment of HPV-related diseases is focused on the elimination of clinical and subclinical manifestations. In each specific case, treatment is selected individually.

The goal of treating cervical pathology is to eliminate both the pathological process and the underlying changes in the body that caused it. The principles of treatment are as follows:

- 1) ensuring recurrence-free outcomes;
- 2) using organ-preserving treatment methods in women of reproductive age.

One of the important conclusions of the latest American recommendations is that conservative tactics should be taken for CIN I. This is explained by research data showing that: 60% of mild forms of CIN (dysplasia) regress without any treatment, 32% remain unchanged, in 11% there is progression to CIN II, and 1% transform into invasive cancer (Oster A. G.). These findings also apply to HPV-positive women (Syrjanen K. J.).

The treatment of HPV infection in women should be carried out simultaneously in several directions. The goal of therapy is the elimination of the virus from the body, removal of the pathological lesion, and prevention of recurrence. The most prospective comprehensive treatment includes specific antiviral therapy and local impact on the affected areas of the cervix.

Taking into account the possible etiopathogenetic mechanisms of cervical proliferative processes, therapy aimed at eliminating the etiological agents using direct-acting antiviral drugs and by stimulating the body's own defense mechanisms is of great significance. Eliminating the causative factor allows the use of careful treatment methods, which is very important because precancerous conditions and cervical cancer are most often diagnosed in women of reproductive age, many of whom wish to have children in the future.

In the case of HPV infection, interferon preparations can be used.

In recent years, α -, β -, and γ -interferon preparations have been used for the treatment of genital papillomavirus and herpes virus infections, owing to their indirect antiviral, immunomodulatory, and antiproliferative effects (Homberg M. A. et al., 1998; Gross G. et al., 1998; Syed T. A. et al., 1998; Perry C. M. et al., 1999; Petersen C. S. et al., 1999; Samhyn M. A. et al., 2002;

Prylepska V. N. et al., 2003).

As far as inducers of endogenous interferon are concerned, they have several advantages over interferon preparations (Homberg M. A. et al., 1998; Yershov F. I. et al., 2005). In the case of genital papillomavirus and herpes virus infections, interferon preparations increase only the level of α -interferon in the blood and exhibit low immunomodulatory activity.

Interferon inducers, by contrast, exhibit pronounced immunomodulatory effects, stimulate the production of α -, β -, and γ -interferons, and restore the balance between pro-inflammatory (interleukin-2, tumor necrosis factor- α) and anti-inflammatory (interleukin-10) cytokines [11].

However, complete eradication of the virus using interferons and their inducers is not possible, since the imbalance of cellular immunity in HPV infection is supported by such data as the combination of spontaneous regression of hyperplastic manifestations of HPV with infiltration of surrounding tissues by lymphocytes and macrophages. The results of some studies indicate a connection between HPV elimination from the body and the appearance of T-helpers responding to the C-terminal domain of the viral E2 protein.

One of the most prospective approaches in the treatment and secondary prevention of HPV infection is the use of direct-acting antiviral agents – natural flavonoids (derived from a mixture of *Herba Deschampsia caespitosa* L. and *Herba Calamagrostis epigeios* L.), which simultaneously act as immunomodulators, activate apoptosis, and exert an antiproliferative effect on the cells. It should be emphasized that due to their ability to directly affect the activity of virus-specific enzymes, such as DNA and RNA polymerases, the use of this group of medicinal products exhibits a pronounced direct virostatic effect on both DNA and RNA viruses, including human papillomavirus [16–18].

Simultaneous complex systemic and local use of natural flavonoids can be used for the successful treatment of CIN I and CIN II without surgical intervention [19, 20].

Combined systemic and local administration of flavonoids and interferons in pre- and postoperative treatment (LEEP, cryo- or laser coagulation, radio wave ablation) is undoubtedly an effective preparatory approach aimed at reducing the recurrence rate [11] and preserving women's reproductive health.

Local treatment involves the removal of the affected epithelial areas using various methods:

- *Cryodestruction*, radio wave method, laser radiation, diathermal excision, or conization.

Surgical treatment means the removal of affected areas. It is mainly used in the case of large tissue lesions or when malignant formations are suspected.

- *Cryodestruction involves freezing* the neoplasms with liquid nitrogen.

The effectiveness of this method ranges from 54.8% to 75%. Such relatively low effectiveness is due to the viability of the virus even after freezing. Cryotherapy is non-toxic, but it may cause pain during tissue “thawing”. Side effects may include blisters and ulcers. When performing cryotherapy on the cervix, deep tissue damage rarely occurs that may lead to scarring deformity and later may manifest as stenosis, atresia of the cervical canal, or cervical dystonia during childbirth.

- *Radio wave coagulation* is used to remove condyloma acuminatum.

A radio wave knife is used for this procedure. Applying radio-surgical techniques, a wide range of cervical excisions can be performed, including LLETZ (Large Loop Excision of the Transformation Zone). The cutting effect is achieved by heat released during tissue resistance to high-frequency radio waves. These waves “vaporize” the cells as they come into direct contact with the electrode. Loop and scalpel electrodes are used for excision, cutting, and tissue “smoothing”; ball electrodes are used for coagulation. With the help of radio-surgical method, conization of the cervix can be also performed. Most procedures are performed under local anesthesia. The advantages of radio-surgical intervention include: speed of treatment, absence of tissue charring, elimination of difficulties during tissue dissection, no bleeding or pain during the procedure. This treatment method does not cause scab or scar formation, twice reduces epithelialization time, and preserves the architecture of the cervix. The effectiveness of radio-surgical treatment makes on average 86% – 90%.

- *Laser radiation* – the removal of neoplasms using a laser.

This is the only procedure suitable for removal of condylomas in pregnant women.

However, treatment using this method does not exclude the possibility of scar formation.

The use of laser is considered a fairly effective and safe treatment method. The main advantages of CO₂ lasers are: controlled vaporization of biological tissues without necrosis of adjacent areas, absence of tumor cell spread during the procedure, enhanced regenerative capacity of laser wound edges. Among the side effects, intraoperative bleeding should be noted.

Healing of laser wounds occurs by primary intention with minimal scarring.

The effectiveness of laser use in the combined therapy of HPV infection of the cervix is 86–96%. However, one of the important disadvantages of laser therapy is the risk of recurrence in the presence of a type 3 transformation zone, because the laser beam does not affect pathological processes located within the cervical canal.

- *Diathermo-coagulation* is a method for removing condylomas using high-frequency radiation or an electrosurgical knife. Diathermo-conization is one of the most radical methods for removing affected areas. Its effectiveness ranges from 76.7% to 90%. However, the high traumaticity of the procedure and the frequent complications (such as cervical canal stenosis, endometriosis, injury of the uterine isthmus due to deep tissue damage, and subsequent reproductive dysfunction) limit its use in young women.

• *Hysterectomy*. • Indications: CIN III localized in the cervical canal; age of the woman – over 50 years; technical impossibility of performing conization due to anatomical features; combination with uterine fibroids or ovarian tumors; recurrences after excisional treatment methods. If the process spreads to the vaginal fornix, extirpation of the uterus with the upper third of the vagina is indicated.

Post-treatment follow-up for benign conditions is carried out for 3 months, during this period it is recommended to limit sexual activity and use local suppositories with methyluracil, hyaluronidase, or thiotriazoline [15]. After the treatment of CIN, follow-up continues for up to 2 years.

Postoperative care plays a significant role in the final outcome of surgical treatment.

Excision is a common cause of stenosis and other complications. This is due to the fact that the healing phase of the cervix is the same as in any other part of the human body. It is important to understand that after surgical treatment, the wound becomes infected with anaerobic flora. During the regeneration phase, it is necessary to support cellular proliferation.

In order to conduct relapse prevention therapy and promote the restoration and strengthening of the immune system, which is especially important in combating HPV, it is necessary to prescribe drugs with direct antiviral action (flavonoids).

The management of CIN during pregnancy is a significant issue.

American guidelines and WHO recommendations emphasize that during pregnancy only invasive cancer should be treated, so the primary task of the physician is to exclude the diagnosis of invasive cancer in the patient.

However, treating women during pregnancy presents certain difficulties due to the inability to use many classes of medications that are either prohibited or not recommended for use in pregnant women. At the same time, drugs that combine both direct antiviral action and immunomodulatory activity, and are approved for use during gestation period (such as natural flavonoids), can both increase the effectiveness of treating various virus-related conditions, and help reduce the frequency of obstetric and perinatal complications in such women.

Opportunities for cervical cancer prevention through HPV vaccination

To prevent HPV, it is necessary to create conditions in the human body for the development of specific long-lasting circulating antibodies against HPV, which is possible only through vaccination. Primary prevention of cervical cancer involves measures targeting individuals who do not show signs of the disease, with the aim of preventing its development in the future.

A classic example of primary prevention of cervical cancer is vaccination of adolescent girls before the onset of sexual activity.

One of the effective methods of combating HPV is vaccination. Currently, two types of HPV vaccines are used in clinical practice, and a third one – the onavalent vaccine – has recently become available. The mechanism of action of these vaccines involves the activation of cellular immunity as well as activation of IgG class antibodies [16]. They are made from purified structural proteins L1, which form HPV type-specific empty shells or virus-like particles (VLPs): HPV-6, HPV-11, HPV-16, HPV-18 – quadrivalent vaccine, HPV-16, HPV-18 – bivalent vaccine, HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52, HPV-58 – onavalent vaccine.

Vaccines do not contain live biological products or viral DNA (they are non-infectious).

HPV vaccines are intended for disease prevention, not for treatment. It should be noted that the vaccine does not protect against all HPV types. This means that it will not prevent the development of all cases of cervical cancer or genital warts.

Approximately 30% of cervical cancer cases will not be prevented by vaccination (regular Pap tests are still necessary), as the vaccine does not affect other oncogenic strains.

The vaccine does not prevent the development of genital warts in about 10% of cases, and does not protect against other sexually transmitted infections.

Secondary prevention of cervical cancer involves early detection and treatment of women with precancerous changes of the cervix, in order to prevent the subsequent development of invasive cancer. Tertiary prevention of cervical cancer is comprehensive or combined treatment, which includes surgery in combination with radiation therapy and chemotherapy.

References:

1. Human papillomavirus infection // Introduction of HPV vaccines in European Union countries – an update. – 2012.
2. Bruni L., Diaz M., Castellsague X. Cervical Human Papillomavirus Prevalence in 5 Continents: MetaAnalysis of 1 Million Women with Normal Cytological Findings // J. Infect. Dis. – 2010. – Vol. 202 (15 December). – P. 1790.
3. Cutts F. T., Franceschi S., Goldie S. Human papillomavirus and HPV vaccines: a review // Bulletin of the World Health Organization. – 2009.
4. Atkinson W., Wolfe S., Hamborsky J. Human Papillomavirus // Epidemiology and Prevention of Vaccine Preventable Diseases [The Pink Book: Course Textbook]. – 12th Edition Second Printing, 2012.
5. Atkinson W., Wolfe S., Hamborsky J. Human Papillomavirus // Epidemiology and Prevention of Vaccine Preventable Diseases. [The Pink Book: Course Textbook]. – 12th Edition Second Printing, 2012.
6. Herbert A., Bergeron C., Wiener H. et al. European guidelines for quality assurance in cervical cancer screening: recommendations for cervical cytology terminology. Cytopathology 2007; 18: 213–219.
7. Arbyn M., Anttila A., Jordan J. et al. European guidelines for quality assurance in cervical cancer screening Second Edition, 2008.
8. Comprehensive Cervical Cancer Control: A guide to essential practice, 2010, World Health Organization, www://whqlibdoc.who.int/publications/2006/9241547006_eng.pdfua=1.
9. European guidelines for quality assurance in cervical cancer screening: recommendations for collecting samples for conventional and liquid-based cytology (2007).
10. Berzolla C. E. Dysplasia and malignancy in endocervical polyps / C. E. Berzolla // J Womens Health (Larchmt). – 2007. – Nov. 16 (9). – P. 1317–1321.
11. Джей Наомі. Папіломавірусна інфекція у жінок / Джей Наомі, Мосціцкі Анна-Барбара // Жінка і здоров'я. – 2010. – Т. 2. – 196.
12. Патологія шийки і тіла матки: посібник для лікарів акушерів-гінекологів, онкологів, цитологів, гістологів, викладачів і студентів мед. ін-тів / за ред. О. В. Коханевич. – Ніжин: Гідромакс, 2009. – 350 с.
13. Тімушш Е., Адамс К. Шийка матки. Цитологічний атлас / Е. Тімушш, К. Адамс: пер. з англ. за ред. Н. І. Кондрикова. – М.: Практична медицина, 2009. – 251 с.
14. Данилова Н. В. Пухлини шийки матки. Морфологічна діагностика і генетика: Посібник для лікарів / Н. В. Данилова, Ю. Ю. Андрєєва, Л.Е. Завалішина, Т. В. Кекеєва, П. Г. Мальков, Г. А. Франк; під ред. Ю. Ю. Андрєєвої, Г. А. Франка. – М.: Практична медицина, 2012. – 116с.

15. Волошина Н. М., Волошин М. А. Скринінг і профілактика раку шийки матки. – Запоріжжя: Друкований світ, 2010. – 155 с.
16. Войтенко Г. М., Коханов І. В. Фармакоекономічне обґрунтування застосування препарату Протефлазид® у терапії папіломавірусної інфекції // «Рецепт». – 2016. – Т. 19, №3.
17. Zaporozhan V. N., Marichereda V. G., Dimcheva L. I. Застосування флавонових глікозидів у терапії генітальної папіломавірусної інфекції у жінок // Application of flavonivyh glycosides papilomavirusnoy therapy of genital infection in women. Journal of Health Sciences. 2014; 4 (12) : 93–104. ISSN 1429–9623 / 2300–665X.
18. Особливості перебігу вагітності та пологів у жінок з багатопліддям / Б. М. Венцківський, І. В. Поладич, В. В. Біла, О. Ю. Костенко, К. С. Казидуб // Медицина транспорту України. – 2015. – №3–4. – С. 8–15.
19. Ventskivsky B. Proteflazid® in the treatment and prevention of human herpesvirus infection in pregnant women: a meta-analysis of clinical trials results / B. Ventskivsky, O. Grynevych, MD, V. Mikhailov, M. Lesnikova, L. Yaschenko, T. Broun // American Journal of Science and Technologies. – 2016. – №1 (21). – January-June. – P. 983–1002.
20. Годлевська Н. А. Ефективність системного та місцевого застосування препарату Протефлазид® у лікуванні патології шийки матки, спричиненої папіломавірусною інфекцією / Н. А. Годлевська, А. В. Старовір // Здоров'я жінки. – 2012. – №3 (69). – С. 80–83.
21. Годлевська Н. А. Клінічна ефективність місцевого застосування вагінальних супозиторіїв Протефлазид® у лікуванні цервікальної інтраепітеліальної неоплазії легкого та помірного ступенів, зумовленої вірусом папіломи людини / Н. А. Годлевська, А. В. Старовір // Здоров'я жінки. – 2017. – №2 (118). – С. 55–60.

Reference: Zaporozhan V. M., Kaminskyi V. V., Svintsitskyi V. S. et al. *National Consensus on the Management of Patients with Cervical Intraepithelial Neoplasia Caused by Human Papillomavirus Infection // Women's Health, – 2017. – №7 (123). – pp. 16–24.*



The project of the Scientific and Manufacturing Company «Ecopharm» LTD ***Innovative direct action anti-HPV agent for cervical cancer prevention*** was awarded with the Seal of Excellence certificate from the European Commission on February 18, 2019.

On October 9, 2019, another project of the company SMC «Ecopharm» LTD titled ***Innovative natural direct action antiviral agent for ARI treatment*** also received the Seal of Excellence certificate from the European Commission.

The leader of the recognized projects is O. Y. Hrynevych, Doctor of Medical Sciences, Honored Scientist and Technician of Ukraine, Laureate of the Prize of the Cabinet of Ministers of Ukraine.

The "Seal of Excellence" is a mark of quality awarded by the European Commission to researchers for project proposals submitted for funding under the Horizon 2020 programme, which is the EU Framework Programme for Research and Innovation. Each year, thousands of organizations across Europe apply to the Horizon 2020 programme with their proposals. The European Commission manages one of the most comprehensive evaluation systems in the world, using an international group of independent experts and evaluates proposals based on defined project indicators: excellence, impact, quality and efficiency of implementation.

Awarding with the SEAL OF EXCELLENCE certificates indicates that the projects ***Innovative direct action anti-HPV agent for cervical cancer prevention and Innovative natural direct action antiviral agent for ARI treatment***, submitted by SMC «Ecopharm» LTD for the competition, were evaluated by an international group of independent experts as "high-quality project proposals in a highly competitive evaluation process", and also that the projects passed the strict evaluation criteria of the Horizon 2020 programme based on such indicators as excellence, impact, quality and efficiency of implementation, and, thus, the stated topics are of exceptional relevance to the international community.

For its part, the medicinal product PROTEFLAZID®, presented in both projects as a natural broad-spectrum antiviral medicinal product, attracted attention of European experts, as it is a promising antiviral drug for the prevention and treatment of dangerous viral infections of today. Its use in practical medicine is considered prospective for the future in maintaining sustainable human health.

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The project proposal 859790, UniVirTTM

Innovative direct action anti-HPV agent for cervical cancer prevention

Submitted under the Horizon 2020's SME instrument phase 2
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in the area of EIC-SMEInst-2018-2020

SME instrument

by

Scientific & Manufacturing Company Ecopharm Ltd.

9V Stepana Bandery Str.
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following evaluation by an international panel of independent experts

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* This means passing all stringent Horizon 2020 assessment thresholds for the 3 award criteria
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Corina Cretu,
Commissioner for
Regional Policy

Carlos Moedas
Commissioner for Research
Science and Innovation

Brussels, 18/02/2019



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The project proposal 890043, ARItreat

Innovative natural direct action antiviral agent for ARI treatment

Submitted under the Horizon 2020's SME instrument phase 1
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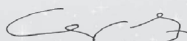
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Commissioner for
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