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Therapeutic efficacy of Proteflazid® suppositories in patients with urogenital viral and bacterial infection

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The article presents the materials of the phase-II clinical study of the comparative evaluation of the efficacy and tolerability of Proteflazid® suppositories and **Proteflazidum® drops in patients with urogenital viral and bacterial infection. The high efficacy of Proteflazid® suppositories, comparable with that of the drug Proteflazidum® in drops (in the form of vaginal tampons) has been demonstrated. Based on the presented data it was concluded that the drug Proteflazid®** suppositories **can be recommended for implementation in the healthcare practice as an effective and safe drug for treating patients with the mentioned pathology.**

Keywords*: *Proteflazid® suppositories, ***Proteflazidum®*** drops, urogenital viral *and bacterial infection, Herpes simplex virus, chlamydia*.

According to the WHO experts, 20 million new cases of herpes virus infection are registered each year in the developed countries [1, 5]. Chlamydia is also one of the most prevalent sexually transmitted diseases. No summary data are available for the incidence in Ukraine, but in the USA, over 4 million new cases of infection are registered per year [2]. The presence of chlamydia increases the risk of HIV infection and other sexually transmitted diseases (Siewert K. et al., 2005; Workowski, K.A., Berman S.M., 2006).

In this regard, the issue of the development and improvement of the existing methods of pathogenetic therapy in the treatment of mixed viral and bacterial infections of the urogenital tract becomes especially urgent. Emergence of resistant herpes infection and persistent chlamydial infection forms has been reported [3, 7]. Therefore, rational etiological and immune-rehabilitation therapies with an effect on the local immunity of mucous membranes are the necessary stages, which allow to reliably prevent the disease chronicity and the persistence of infection. One of such new domestic drugs that has recently gained recognition among professionals and population is Proteflazid®.

The choice of a dosage form of suppositories is not accidental, since it allows to successfully apply Proteflazid® at the site of infection in patients of different age and functional status.

The choice of the drug for treatment was conditioned by the following characteristics of Proteflazid®. The drug active substance (flavonoids) inhibits the synthesis of DNA- and RNA-viruses in the infected cells due to the inhibition of activity of virus-specific enzymes, such as RNA-, DNA-polymerase, thymidine kinase and reverse transcriptase; it also has immunotropic properties. It has been established that the active substance is able to activate the synthesis of endogenous alpha- and gamma-interferons to the level of physiological activity (without the development of refractoriness), which increases the non-specific resistance to viral and bacterial infections. Clinical studies of Proteflazid® suppositories have established that they restore the protective function of the mucous membrane of the cervix thanks to the normalization of local immunity factors (sIgA, lysozyme, and complement C3). In case of genital herpes, the drug prevents the formation of new rash elements, reduces the risk of dissemination and visceral complications, and accelerates healing of the lesions. In case of vaginosis, vaginitis, and inflammatory diseases of the cervix, the drug helps to restore the local immunity and eliminate the pathogen faster and more effective. Proteflazid® shows antioxidant properties inhibiting the course of free-radical processes, thereby preventing the accumulation of products of lipid peroxidation, and enhancing the antioxidant status of the cells. The drug is a modulator of apoptosis, enhancing the effect of apoptosis-inducing substances, namely by activating caspase-9, which facilitates more rapid elimination of the virus-infected cells and primary prevention of chronic diseases associated with latent viral infections.

The drug Proteflazid®, suppositories, has been tested in the course of tolerability studies (phase I). Besides, the safety and efficacy of the drug active substance have been proven by wide clinical use of the drug Proteflazidum®, drops, since 2001.

The selection of the drug for basic therapy is based on the Orders of the Ministry of Health of Ukraine No. 312 dated 08.05.2009 and No. 286 dated 07.06.2004, regulating the primary care of patients with dermatovenereologic pathology. According to the Orders, the treatment program applied for chlamydia infection includes the use of azithromycin in standard dosages. Recommendations regarding the supplementation of etiotropic therapy with immunocorrecting drugs in the treatment of herpesvirus pathology and chlamydia are included in the treatment programs legally used abroad [4, 8].

Study objective: to evaluate the efficacy of the combination of Proteflazid®, suppositories ("Pharmex Group" LLC, Ukraine), with the drug Azimed – coated tablets 500 mg each (Arterium Corporation, Ukraine) in comparison with the combination of Proteflazidum®, drops, (PJSC "Phytopharm", Ukraine) with the drug Azimed – coated tablets 500 mg each (Arterium Corporation, Ukraine) in patients with urogenital viral and bacterial infections.

Table 1

Distribution of subjects by gynecological history

Parameter	Main group, n=35		Control group, n=35		Significance of differences*
	Absolute number	%	Absolute number	%	
Deliveries	10	28.6	14	40.0	0.4500
Abortions	15	42.9	12	34.3	0.6234
Miscarriages	9	25.7	7	20.0	0.7759
Menstrual disorders	2	5.7	1	2.9	1.000

*Note. * — The estimation was carried out using the chi-square test with Yates' correction for continuity in combination with Fisher's exact test.*

MATERIALS AND METHODS

Study objectives:

– To study the therapeutic efficacy of the study drug Proteflazid®, suppositories ("Pharmex Group" LLC., Ukraine), in patients with urogenital viral and bacterial infection;

– To study the therapeutic efficacy of the study drug Proteflazidum®, drops (produced by PJSC "Phytopharm", Ukraine), in patients with urogenital viral and bacterial infection;

– To compare the results of treatment in the main and control groups in order to evaluate the efficacy of the treatment.

The study involved 70 women aged from 18 to 51 years, which were divided into two groups according to the randomization scheme – the main and control groups at the ratio of 1:1. Patients of both groups were treated with Azimed – coated tablets 500 mg each (Arterium Corporation, Ukraine) as basic therapy. The drug was taken orally according to the scheme: the 1st day of treatment – 1.0 g, the 2nd – the 5th day of treatment – 0.5 g per day.

The patients of the I (main) group were additionally prescribed with the study drug Proteflazid®, suppositories ("Pharmex Group" LLC, Ukraine), for 14 days against the background of basic therapy.

The patients of II (control) group were prescribed with the reference drug Proteflazidum®, drops (PJSC "Phytopharm", Ukraine), 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 saline sodium chloride solution. The time of vaginal tampon exposure was 30-40 minutes; the procedure was to be performed 2 times a day for 14 days.

The control of indicators was carried out before the study beginning, at the end of the course of therapy and 4 weeks after the treatment completion. The conclusion about the efficacy was made based on the evaluation of the analysis results of smears from the epithelium of the vaginal/cervical mucosa by PCR for the presence of chlamydial DNA and the DNA of HSV-1, HSV-2 at the end of the treatment course and follow-up period. The drug was considered effective when no *Chlamydia trachomatis* DNA was found in the specimen taken from the lesion focus at two-fold laboratory inspection by PCR, as well as when the level of HSV DNA decreased by an order or greater.

STUDY RESULTS AND DISCUSSION

At the screening phase, gynecological history data and patient's compliance with the study inclusion criteria were evaluated. The distribution of patients by gynecological history is presented in Table 1.

Abortions, miscarriages, and menstrual cycle disorders prevailed in the gynecological history. No statistically significant differences were identified between the groups.

Seventy women enrolled in the study received a course of treatment within 14 days. At the end of the course of treatment and in 4 weeks after the completion of the course of treatment, the detection of chlamydial DNA and HSV DNA in smears from the epithelium of the vaginal/cervical mucosa was repeated, as well as the evaluation of HSV markers (IgG, IgM).

Assessment of the proportion of subjects in each of the groups, for whom *Chlamydia trachomatis* DNA was determined in smears from the epithelium of the vaginal/cervical mucosa at two-fold laboratory inspection is presented in Fig. 1.

Chlamydia DNA was screened in 100% of patients of each of the

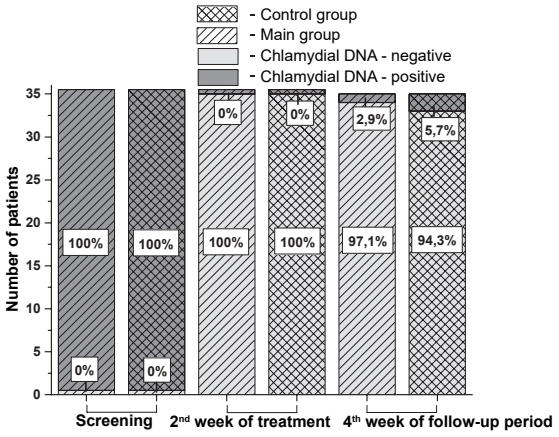

Fig. 1. Detection of chlamydia DNA over time

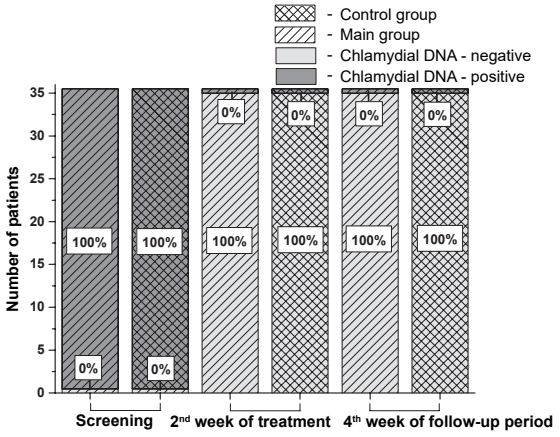

Fig. 2. Detection of HSV DNA over time

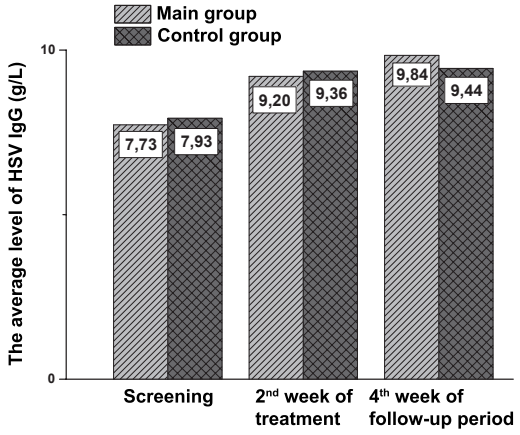

Fig. 3. Dynamics of the "IgG" indicator, g/L

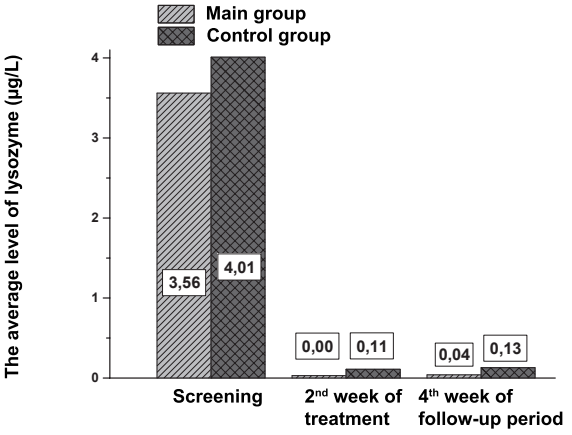

Fig. 5. Dynamics of the "Secretory IgA" indicator, µg/L

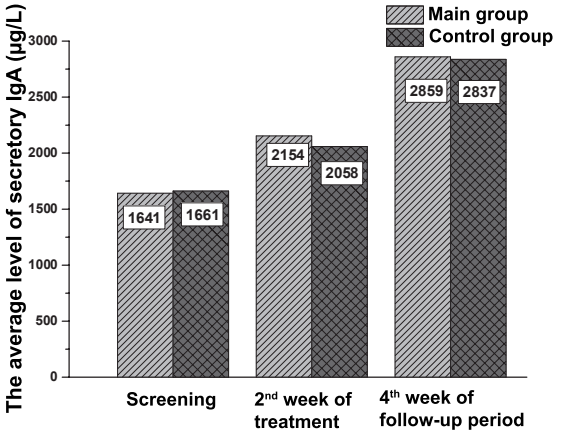

Fig. 4. Dynamics of the "IgM" indicator, g/L

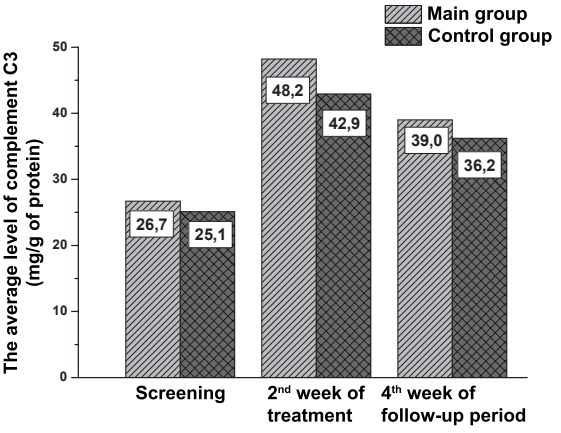

Fig. 6. Dynamics of the "Lysozyme" indicator, µg/L

groups, which was the eligibility criterion for participation in the study. At the end of the 14-day treatment course, no chlamydia DNA was detected in any of the cases in each group.

HSV DNA was revealed in smears from the cervical epithelium in all examined women of both groups before the treatment (which also meets the eligibility criteria).

By the end of the course of treatment, and also by the end of the 4-week follow-up period, no HSV DNA was detected in any of the cases (Fig. 2).

According to the data given, a conclusion has been made about the significant decrease in the level of DNA markers of *Chlamydia trachomatis* and HSV in both groups.

HSV IgG and IgM assessment data in groups over time are shown in Fig. 3 and Fig. 4.

The presented data allow us to note a significant decrease in the level of HSV IgM serological markers and increase in the level of HSV IgG in both groups.

At the end of the course of treatment and 4 weeks after the end of treatment, a reassessment of local immunity indicators (secretory IgA, lysozyme, complement C3) was performed. Data on the evaluation of the local immunity indicators over time are shown in Fig. 5-7.

A significant increase in the level of secretory IgA and the level of lysozyme was observed on the 2nd and 4th week in both groups in comparison with the status at the screening. Level of complement C3 significantly increased by the end of the treatment in the main group and returned to the baseline by the end of the follow-up period. In the

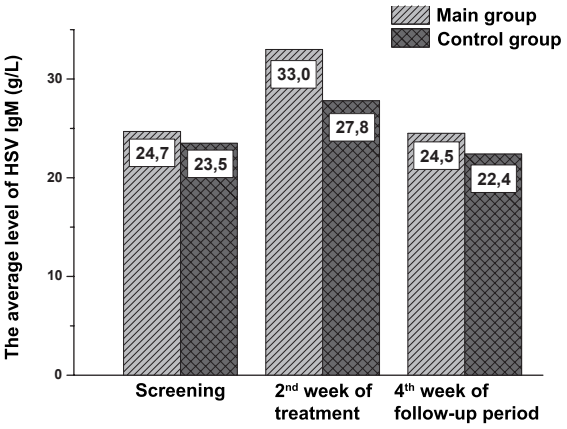

Fig. 7. Dynamics of the "Complement C3" indicator, µg/g of protein

control group, no significant changes in the level of the complement C3 were noted.

Conclusion on the efficacy of the drug Proteflazid®, suppositories, in comparison with the drug Proteflazidum®, drops, in the form of vaginal tampons with a solution of the drug in patients with urogenital viral and bacterial infection was made according to the confidence interval approach. The results of the calculations are given in Table 2.

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Based on the data presented, the treatment was considered to be effective in 34 (97.1%) patients of the main group and in 33 (94.3%) patients of the control group.

No serious adverse reactions or events were registered during the study. None of the patients had to terminate the participation in the study early due to undesirable events.

Table 2

95% CI limits for the difference between the proportions of positive results

Statistical parameter	Value
Probability of type I error, α	0.025
Percentage point of the standard normal distribution, α	1.96
Zone of efficacy, δ (%)	-20%
Proportion of positive outcomes for the main group, %	97.1
Size of the main group	35
Proportion of positive outcomes for the control group, %	94.3
Size of the control group	35
Proportion difference, %	2.8
Standard difference error	5.523
95% confidence interval lower limit	-16.53
95% confidence interval upper limit	5.13

The compared drugs did not exhibit any negative effects on the blood pressure, heart rate, and body temperature: at the end of the clinical study, no negative changes in these indicators compared with the baseline before treatment were observed in patients of any of the groups. Laboratory indicators also did not deteriorate in any of the cases. No cases of exacerbation of the existing chronic diseases, anaphylactic reactions, delayed-type reactions or critical changes in the hemodynamic and basic laboratory indicators were recorded. Based on the data presented, the tolerability assessment of the studied drugs was performed (Table 3).

It is necessary to focus on a number of points of the given study. Thus, chlamydia DNA was detected at screening in 100% of subjects of each of the groups, which was the criterion for selecting subjects for participation in the study. At the end of the 14-day course of treatment, chlamydia DNA was not detected in any of the cases in each of the groups. Newly detected chlamydia DNA at the last examination of patients after the 4-week follow-up period (in 1 patient of the main group and in 2 of the control group) can be evidence of reinfection.

Observation of the local immunity indicator dynamics in the examined groups showed positive changes of the parameters of secretory IgA, lysozyme, and complement component C3, which indicates positive effect of the treatment on the local immunity. Similar changes were reported by other authors [6]. At the same time, the mentioned tendency was less expressed in the control group. This fact, apparently, indicates a more pronounced effect of Proteflazid in the form of suppositories on the state of local immunity in comparison with drops.

CONCLUSIONS

1. The study drug Proteflazid®, suppositories, is highly effective in patients with urogenital viral and bacterial infection of mixed etiology (Herpes simplex viruses and chlamydia). According to the criteria specified in the study protocol, the efficacy of the treatment was 97.1%.

2. On the basis of the data obtained during the study it was concluded that the study drug Proteflazid®, suppositories, is not inferior in efficacy to the drug Proteflazidum®, drops, in the form of vaginal tampons with the drug solution at local application.

3. In both groups, a significant reduction of HSV DNA viral load (detected in all patients at the screening phase) in comparison to the baseline was observed. After completing the 14-day course of treatment, as well as after the 4-week follow-up period, no HSV DNA in the smears from the epithelium of mucous membranes of the vagina/cervix was detected in any of the cases.

4. Both groups had a significant reduction of chlamydia DNA prevalence compared to the baseline. After the end of the 14-day course of treatment, no chlamydia DNA was detected in any of the cases, at the end of the 4-week follow-up period, chlamydia DNA was detected in 1 patient of the main group and in 2 of the control group.

5. Additional evidence of efficacy is a significant increase of the local immunity indicators in both groups. In particular, the level of secretory IgA and lysozyme has increased already by the 14th day of treatment, remaining significantly high throughout the entire follow-up period (for secretory IgA: 1641.9 μg/L - on screening; 2154.2 μg/L - on the 14th day; 2859.3 μg/L - by the end of the 4-week follow-up period; for lysozyme: 26.7 μg/L - on screening; 48.2 μg/L - on the 14th day; 39.0 μg/L - by the end of the 4-week follow-up period); The complement C3 level increased in the main group by the 14th day of treatment and returned to the baseline by the end of the 4-week follow-up period (24.7 μg/g of protein - on screening; 33.0 μg/g of protein - on the 14th day; 24. μg/g of protein - by the end of the 4-week follow-up period for complement C3).

6. A decrease in the level of HSV IgM serological markers was observed in both groups of subjects: after the completion of the 14-day course of treatment - from 3.56 to 0.00, and after the completion of the 4-week follow-up period – from 0.11 in the main and 4.01 in the control group to 0.01 and 0.13, respectively.

7. On the basis of the presented data, Proteflazid® suppositories can be recommended as an effective and safe antiviral agent for the treatment of patients with mixed urogenital viral and bacterial infection.

Table 3

Results of the drug tolerability assessment

Tolerability	Main group, n=35		Control group, n=35	
	Prevalence	Proportion, %	Prevalence	Proportion, %
Good	35	100.0	35	100.0
Satisfactory	0	0	0	0
Unsatisfactory	0	0	0	0

Therapeutic efficacy of Proteflazid, suppository among the patients with genitourinary viral-bacterial infection
V.O. Benyuk, O.Y. Grynevych, I.A. Usevich, I.S. Sikachova, M.V. Shepetko
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®, suppositories with comparable values for Proteflazidum®, drops (in the form of vaginal tampon with the drug solution). Presented data allowed to draw conclusion that Proteflazid®, suppositories can be recommended for implementation in clinical practice as a drug with high efficacy and good tolerability for the treatment of genitourinary viral-bacterial infection.
Key words: *Proteflazid®, suppository; Proteflazidum, drops; Deschampsia caespitosa L.; Calamagrostis epigeios L.; flavonoids; viral-bacterial mixed infection, Herpes genitalis, Chlamydia*

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