Proteflazid® and local immunity in diseases caused by human papillomavirus, herpesvirus and mixed urogenital infections

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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 aim *of study was to 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).*
Material and methods. *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).*
Results. *Treatment results have confirmed that Proteflazid® contributes to sustainable performance improvement of basic factors of local immunity – sIgA, lysozyme and complement component C_3 in the cervical mucus for all three groups of women.*
Conclusions *Proteflazid® enhances level of local immunity markers (sIgA, lysozyme, C_3 complement component) and improves their ratios. Also it intensifies anticontagious activity of mucosal protection and female reproductive system as whole, during treatment diseases caused by human papillomavirus, herpesvirus and mixed urogenital infections (herpesvirus and chlamydia).*

Key words: *local immunity, papillomavirus, herpes, mixed infection, Proteflazid®*

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Proteflazid® i lokalna odporność na choroby wywołane przez wirusa brodawczaka ludzkiego, opryszczki i mieszanych zakażeń układu moczowo-płciowego

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W artykule przedstawiono wyniki badań wpływu preparatu Proteflazyd®, czopków Proteflazyd®, kropli (w formie tamponów, nasączonych roztworem preparatu) na miejscową odporność kobiecych organów płciowych.
Celem pracy *było zbadanie stanu odporności miejscowej w drogach rodnych kobiet z chorobami przenoszonymi drogą płciową, wywołanych przez wirusa brodawczaka ludzkiego, wirusów opryszczki (typ 1, 2) i mieszaną infekcję (wirusy opryszczki + chlamydia).*
Materiał i metody. *W badaniach brało udział 216 kobiet z wirusowymi i bakteryjnymi zakażeniami przekazywanymi drogą płciową, a w tym z dysplazją szyjki macicy związaną najczęściej z brodawczakiem ludzkim (HPV) (1 grupa), z opryszczką narządów płciowych 1 (HSV-1) oraz typu 2 (HSV-2) (2 grupa), z zakażeniem mieszanym – HSV-1, HSV-2 i chlamydioza (3 grupa).*
Wyniki*. Badania wskazują, że leczenie Proteflazydrm® sprzyjają stabilnemu wzrostowi czynników odporności miejscowej – sIgA, lizozymu, C_3 składnika dopełniacza w śluzie szyjkowym wszystkich trzech grup kobiet.*
Wnioski. *Proteflazid® zwiększa zawartość oraz ulepsza wzajemne proporcje wskaźników odporności miejscowej – sIgA, lizozymu oraz składnika dopełniacza C_3. Wzmacnia ochronę przeciwzakaźeniową śluzu szyjkowego oraz kobiecego układu rozrodczego, ogólnie w leczeniu chorób wywołanych przez wirusa brodawczaka ludzkiego, zakażenie opryszczkowe oraz infekcje urogenitalne (wirusy opryszczki i chlamydiozy).*

Słowa kluczowe: *odporność miejscowa, wirus brodawczaka, opryszczka, infekcja mieszana, Proteflazyd®*

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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 repro-

ductive 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 of ability 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 was to 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 C_3 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 (HSV-1, 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 1 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).

sIgA baselines in subgroups A and B (before treatment) in patients in group 2 with certain disease "Genital Herpes (HSV-1, 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 C_3 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 C_3 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 C_3 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 C_3 before treatment was significantly higher than the conventional norm,

Fig.1a

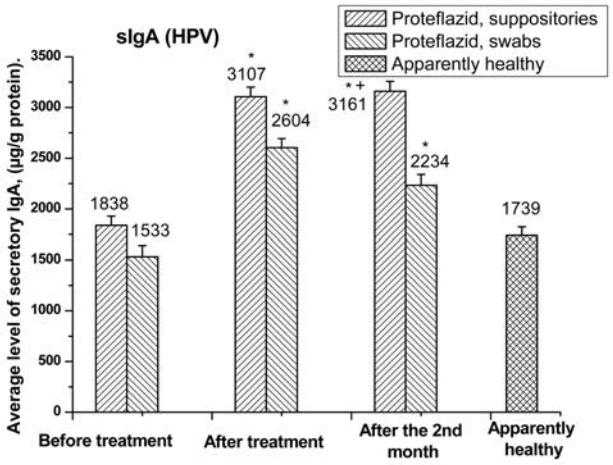

Fig.1.b

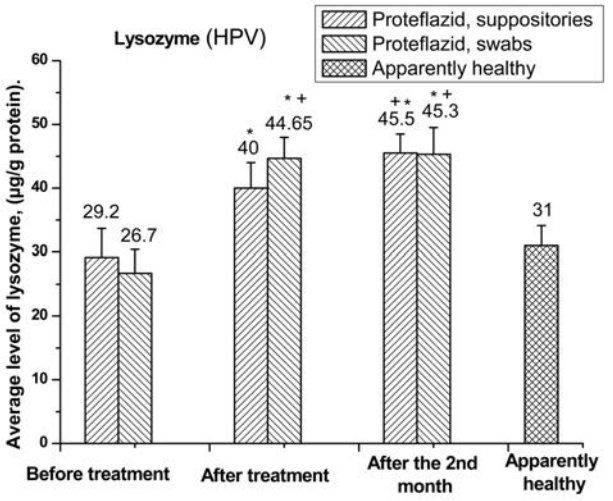

Fig.1c

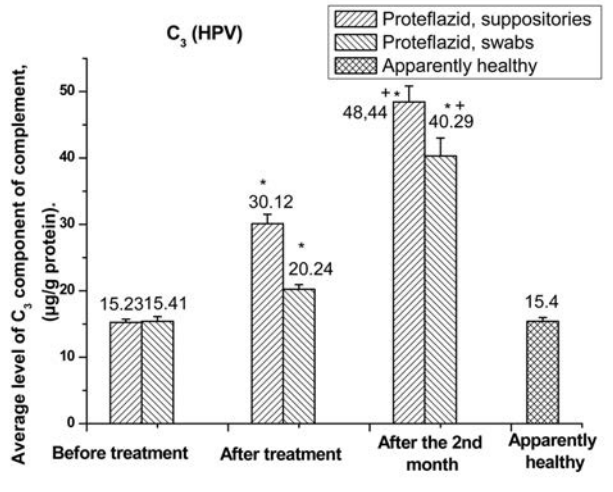

Figure1. Dynamics of sIgA (1a), lysozyme (1b) and complement component C_3 (1c) in patients with "cervical dysplasia epithelium caused by HPV infection", before and after treatment with the drug Proteflazid®, administered in the form of vaginal suppositories and vaginal swabs soaked in the solution of Proteflazid® extract (mean ± SE)

Rycina 1. Dynamika sIgA (1a), lizozymu (1b) i składnika C3 dopełniacza (1c) u chorych z dysplazją nabłonka szyjki macicy spowodowane przez zakażenie HPV zakażenie przed i po leczeniu lekiem Proteflazid® podawanym w postaci czopków dopochwowych i tamponów dopochwowych nasączonych ekstraktem Proteflazid® (średnia ± SE)

* – $p<0.05$ against the level before treatment

\+ – $p<0.05$ against the conventional norm

Fig.2a

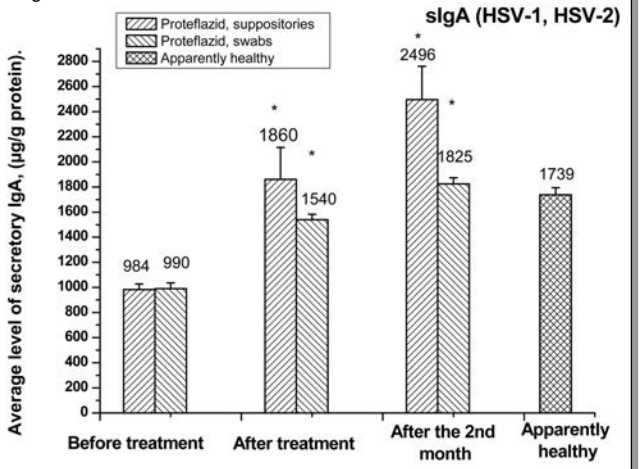

Fig.2b

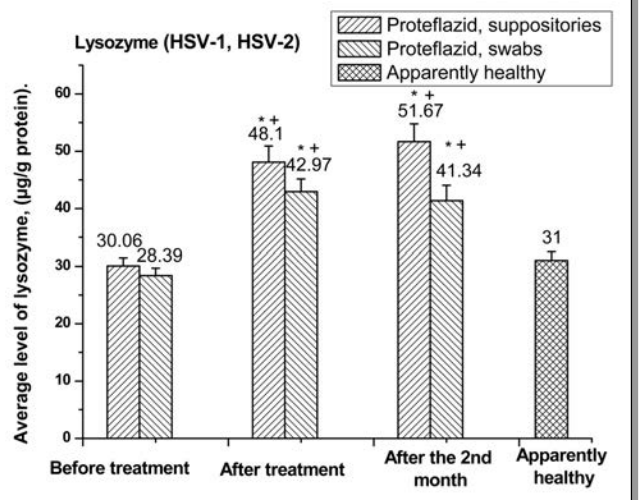

Fig.2c

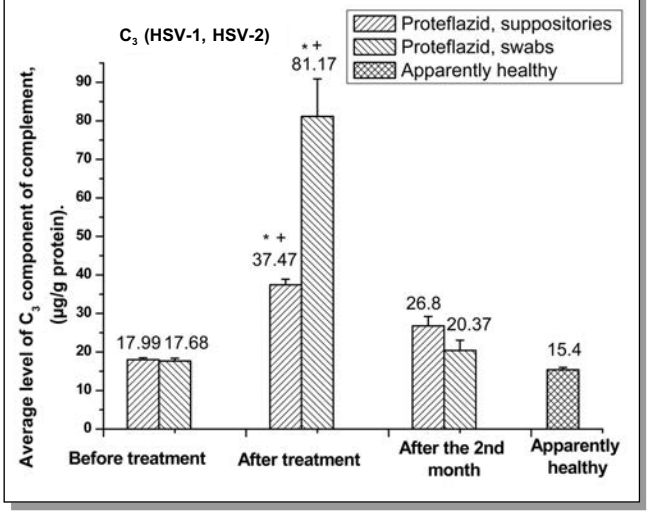

Figure 2. Dynamics of sIgA (2a), lysozyme (2b) and complement component C_3 (2c) in patients with "Genital Herpes (HSV-1, HSV-2) in the exacerbation phase", before and after treatment with the drug Proteflazid®, administered in the form of vaginal suppositories and vaginal swabs soaked in the solution of Proteflazid® extract (mean ± SE)

Rycina 2. Dynamika sIgA (2a), lizozymu (2b) i składnika C_3 dopełniacza (2c) u pacjentek z wirusem opryszczki narządów płciowych (HSV-1, HSV-2) w fazie zaostrzenia przed i po leczeniu lekiem Proteflazid® podawanym w postaci czopków dopochwowych i tamponów dopochwowych nasączonych roztworem ekstraktu Proteflazid® (średnia ± SE)

* – p<0.05 against the level before treatment

+ – p<0.05 against the conventional norm

Fig.3a

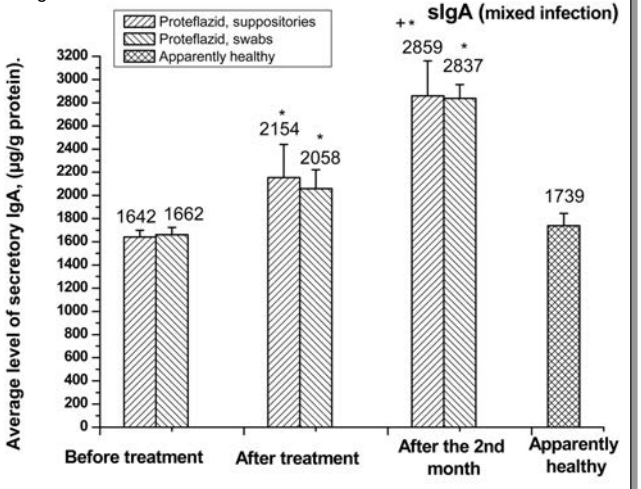

Fig.3b

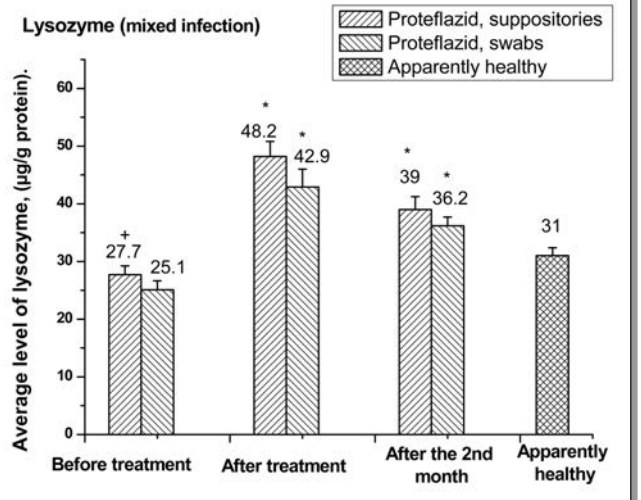

Fig.3c

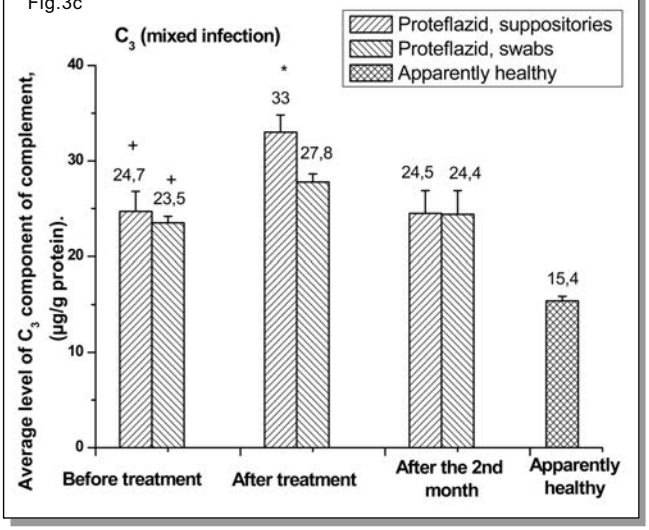

Figure 3. Dynamics of sIgA (3a), lysozyme (3b) and complement component C_3 (3c) in patients with "Urogenital viral and bacterial infection (Herpes simplex viruses and Chlamydia)", before and after treatment with the drug Proteflazid®, administered in the form of vaginal suppositories and vaginal swabs soaked in the solution of Proteflazid® extract (mean ± SE)

Rycina 3. Dynamika sIgA (3a), lizozymu (3b) i składnika C_3 dopełniacza (3c) u pacjentek z wirusowym i bakteryjnym (Herpes simplex wirusy i Chlamydia) zakażeniem układu moczowo-płciowego przed i po leczeniu lekiem Proteflazid® podawanym w postaci czopków dopochwowych i tamponów dopochwowych nasączonych roztworem ekstraktu Proteflazid® (średnia ± SE)

* – p<0.05 against the level before treatment

+ – p<0.05 against 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 anti-infective 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_3 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 C_3 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 C_3 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 C_3 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 C_3 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 macro- and 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 C_3 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 C_3, 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).

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