Immune therapy in the comprehensive treatment of the internal genital organs inflammatory diseases

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Parameters of the local immunity in 48 women patients with tubal-peritoneal infertility against the background of internal genitals chronic inflammation were studied. Changes in the state of the examined women’s local immunity were detected in the course of treatment. The role of immune therapy (Laferobion) in the treatment of pelvic inflammatory diseases has been shown.

Keywords: inflammatory diseases of internal genital organs, infertility, local immunity, immunotherapy.

Inflammatory diseases of the pelvic organs (PID) occupy one of leading places in the structure of gynecological pathology, they are diagnosed in 60-65% of all gynecological patients [1, 2]. The peak incidence occurs between the ages of 15-24 [3]. 2/3 of the PID cases are not recognized [3].

In recent years, the spread of PID ever more negative role is increasingly played by social and behavioral factors that reduce the immune defense of the body and predispose it to the spread of infections [1]. The social factors include chronic stress, low standards of living (under- and poor nutrition), chronic alcoholism, drug abuse; the behavioral factors are early sexual activity, more frequent sexual intercourses, a large number of sexual partners, non-traditional forms of sexual contacts (oral-genital, anal), intercourse during menstruation, as well as the partner’s infectious diseases of the genital organs [1, 4].

PID adversely affect the reproductive, menstrual and sexual functions of women [5]. Thus, in 17-20% of patients, the acute process becomes chronic with the development of adhesions, persistent pain, ovulation disorders, 18% of them will sooner or later develop an ectopic pregnancy [5]. According to the WHO data, untreated gonococcal or chlamydial infection in 40% of women lead to the development of PID, with every 4th woman developing in the future such a complication as tubal infertility (WHO, 2006) [6]. These figures do not reflect the objective reality, because often patients with the obliterated forms of PID do not seek help in medical facilities.

PID is a kind of an onset for the majority of gynecological diseases. Inadequate treatment of PID leads to a chronic process, forming the basis of pathological conditions that impair the fertile function in women and in some cases require surgical correction. Chronic inflammation in the genitals participates in the formation of pathological processes such as hystery of uterus, endometriosis, endometrial hyperplasia, infertility of various origins, neoplastic diseases of the cervix, as well as functional disorders, violating the physiological course of pregnancy [1]. Therefore the doctor should be aware that future implementation of the patient’s reproductive function depends on the approach and efficiency of their assigned treatment.

It is a proven fact that pelvic inflammatory disease is a polymicrobial infection, representing a mixture of sexually transmitted infections agents, endogenous aerobic and anaerobic micro-organisms [7]. In 19-53% of women with chronic endometritis, opportunistic pathogenic microflora from the uterus is plated (E. coli, Enterococcus, Staphylococcus ep.) [1, 2, 7]. In 20-40% of women with PID, Chlamydia trachomatis is detected. The most common PID pathogens are: Chlamydia trachomatis, M.hominis, Ureaplasma urealyticum, Candida albicans.
Currently, cytomegalovirus (CMV), herpes simplex virus (HSV), human immunodeficiency virus (HIV) are associated with PID, in 15-20% of PID cases a pathogen is not detected. The trigger of PID development is the microbial factor’s impact due to the activation of the opportunistic pathogenic microflora in the vagina or getting an infectious agent from the outside. At this, the vagina biocenosis is important together with local and systemic immunity.

An important component of the vaginal barrier are the immune defense factors. Protective mechanisms at the level of the immune system’s mucous membranes (MALT - Mucosal-Associated Lymphoid Tissue) contribute to the rapid containment of the infectious process. It is achieved by a complex interaction of both non-specific effector mechanisms (opsonocytophagic system) and specific immune response, primarily, the antibodies formation. Neutrophil granulocytes constitute the first line of the non-specific antimicrobial defense, they are the first to be mobilized in the inflammation focus and the pathogens elimination depends in many respects on their phagocytic activity.

When the pathogen overcomes the epithelial barrier, it is found in the subepithelial connective tissue by macrophages, which also have a high phagocytic activity. In addition, the interaction of microorganisms with receptors of macrophages results in the induction and secretion of pro-inflammatory cytokines, providing the development of early inflammatory response. The system of interferons (IFN α-, β-, γ-type) is synthesized by leukocytes and MALT cells in response to the antigen induction. One of the key roles in the non-specific anti-inflammatory protection of mucous membranes, which consists in blocking the adhesion of microorganisms to the barrier’s epithelial cells, neutralizing their biological activity, belongs to secretory immunoglobulin A. The third physiological barrier is the cervix filled with mucous secretion, containing large amounts of lysozyme, secretory IgA and other antimicrobial substances.

Infectious agent

Reducing of immune and nonimmune protective properties of the macro-organism
Dysbiotic states, activation of vaginal OPM (opportunistic pathogenic microflora), its translocation into internal genital organs (IGO)

Alteration (biochemical reaction in the inflammation focus), morphological changes

Immune deficiency
Exudation (reaction of neutrophil granulocytes and macrophages)

Augmentation of disorders in the microcirculation system and homeostasis, failure of the the adaptation and compensation physiological mechanisms, intoxication symptoms

Proliferation (proliferation and migration of fibroblasts)

Regeneration
Incompetent
Complete (due to the cellular proliferation)

Incompleteness of the inflammatory process

Chronic process

Secondary immune deficiency
Complete restoration of the Go structure and functions

Incomplete restoration of the Go structure and functions

**Figure 1. Main stages of the female reproductive system’s inflammatory diseases pathogenesis**

If the local protective physiological barrier is overcome, then the infection generalization is taking place. When the performance of local anti-microbial protection mechanisms is efficient, the infectious process can stop at any stage of its development.

Inflammation is the microorganism’s response to the damage, aimed at eliminating the damaging agent. In the focus of inflammation complex dynamic processes take place, that are a signal to activate various systems of the body as a whole, primarily, the immune system. After the infection penetrating into the body, the immune responses develop with complex cellular interactions. Cytokines (IFN) are the regulators of these interactions.

The pathogenesis of the inflammatory process includes: alteration (damage to tissues and cells), release of mediators (triggers), vascular response with the development of exudation and proliferation (Figure 1).

At chronic recurrent inflammatory diseases there occurs a state of secondary immunodeficiency. [9] The causes of PID chronization is the failure of the body's immunological defense mechanisms against the pathogen’s action. This is due to: the systemic disorders of the immune homeostasis; wasting of immune competent cells; decreased production of humoral factors; lack of affect on the modified tissue reactivity and on the local immunity; presence of antibiotic-resistant superbugs (aerobes, anaerobes: *E. coli, Klebsiela, St.aureus*) [1, 7].

It was I.V. Davydovskiy (1928) who noted that "... the problem of inflammation and immunity are closely related to each other", and nowadays the inflammatory and immune responses are considered to be an indissoluble unity [1]. Active IFN production is a pledge of the organism’s resistance to the emergence of infectious diseases or of rapid localizing the focus of infection in the case of its occurrence [9].

The use of etiotropic antibacterial and chemotherapeutic drugs is often ineffective or produces short-term effects, particularly in the treatment of chronic recurrent forms of diseases. This is due not only to the sensitivity change and the emergence of pathogens resistant to antibiotics used, but also due to the lack of this therapy influence on the modified tissue reactivity and local immunity [9]. Developing against this background the so-called immune reactivity syndrome can lead to long-term persistence of the pathogen and the possibility of subsequent infection by infectious agents of a different nature, since the immune status recovery and microbiocenosis normalization are insufficient or does not take place at all.

Under the conditions of the chronic disease course, accompanied by the systemic impairment of immune homeostasis, immune cells wasting, decreased production of humoral factors, the infection control is often ineffective, even if modern etiotropic agents are used. That is why to improve the efficiency of standard antimicrobial treatment of urogenital infections in the current circumstances it is common to use various immunotropic drugs: interferon and its inducers, interleukins, various immunostimulatory preparations obtained by means of chemical synthesis or being the components of the bacterial cell wall, etc. [11, 12, 13].

The main effects of IFN are:

- Antiviral (inhibits the assembly of the virus subunits, blocks viruses’ budding from the cell membrane);
- Immunomodulatory (activates macrophages, T-cytotoxins, NK cells, suppresses the activity of B-lymphocytes);
- Antitumor.

The effect of interferon on intracellular pathogens has been proved [11-13]. Data obtained *in vitro* [Cell Culture: HeLa (cervical epithelial cells), A549 (epithelial cells of the respiratory tract)] demonstrate that the addition of IFN-a inhibited the bacterial growth...
depending on the dose; IFN-a in the amount of 1000 IU / ml reduced the number of Chlamydiae by 52%; the infectuous ability of *C. trachomatis* was also reduced under the action of IFN-a [14].

The combined use of IFN improves the efficiency of antibiotic therapy in treatment of ureaplasmal and mycoplasmal infections [15]. Data obtained *in vitro* (human fibroblasts cell cultures) indicate that the application of IFN-a inhibited CMV growth slowed by 30-40 times.

The most widely used are the exogenous IFN drugs, it inducers, as well as immune stimulators [11-13]. When prescribing a drug belonging to these groups, differences in the mechanisms of action and in the rate of the immunomodulatory / immunostimulatory effect onset should be taken into account. Inducers stimulate cytokine synthesis (endogenous IFN) in the body, but the above synthesis is naturally higher in healthy and low-damaged cells.

The induction mechanism features of inducers are the slow production of endogenous IFN, the infectious agent frequently multiplies at priority growth rates. The cells’ potential of IFN formulation is rapidly wasting (especially in damaged cells), thus limiting the protecting capacity of the body. There is no definitive data on the targeted impact of inducers on the only one IFN system, one can not say for sure that receiving inducers is quite safe. Morover, many inducers are disintegrated in the body faster than cytokines.

Application of IFN-a drugs to treat chronic PIDis also pathogenetically justified because a number of these diseases agents produce a suppression effect both on the immune system as a whole and on the IFN production, in particular [17]. In the case of treating the infectious and inflammatory diseases of the urogenital tract in women, the local drug treatment is preferable, which becomes possible to achieve using interferon-containing suppositories for rectal or vaginal administration [17]. Drugs introduced into the rectum, can faster and more efficiently express its effect as a result of particularly favorable suction conditions. The distal segment of the digestive tract, due to its anatomical and physiological features (rich venous network), is a unique area of the body where the absorption processes reach a special intensity.

**Aim of the Study:** To assess the effect of the rectal interferon drug form (Laferobion, PAT "BIOPHARMA") on the local immunity parameters in women patients with PID.

**MATERIALS AND METHODS**

During the study, Laferobion drug (rectal suppository) was applied in the doses of 1 000 000 - 3 000 000 IU 2 twice a day for 10 days.

The study involved 48 women with tubal-peritoneal infertility exacerbated by chronic recurrent PID.

The average age of patients with chronic inflammatory diseases of internal genital organs was 33.2 ± 1.2 years of age. Primary infertility was found in 43.7%, secondary infertility - in 56.3%. The infertility duration was 4.3 ± 0.7 years.

Local immunity (in-house data)

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Associations of microorganisms in the analysis of the vaginal discharge and the presence of sexually transmitted infections were identified in all the women. In all the examined patients, species and quantitative composition of microorganisms, causative agents of internal genital organs inflammation, was studied. Detection of HSV and CMV markers (anti CMV and HSV IgM and IgG) in the blood serum was performed by means of enzyme immunoassay using the test kits produced by Diagnostic Prod. Co., US; "Vector-Best", Russia. Isolation of CMV and HSV DNA (PCR) was performed using "AmpliSens" test systems (Russia), detecting of urea/mycoplasmal infection was performed by means of the respective kits. Using the PCR method, C.trashomatis DNA was determined in the cervical canal discharge, where it was detected in 27% of women patients with chronic PID. To quantify lysozyme, levels of G, M, A immunoglobulins in the cervical mucus, the method of simple radial immunodiffusion in gel was used.

RESULTS AND DISCUSSION

Among the examined women patients, 92% were HSV 1,2 seropositive (the active form was found in 12%), 79% of the patients were CMV seropositive (the active form was found in 2%). Opportunistic pathogenic microflora was found in 83% of patients, urea/mycoplasma in 65%. Microorganisms associations were detected in all the women patients surveyed. Study of the local immunity status in the women surveyed indicates its decrease, accompanied by nearly 2-fold reduction of the secretory immunoglobulin A level and lysozyme in the cervical canal as compared with healthy women (Fig. 2). IgA concentration in the cervical mucus was by nearly 50% higher compared to that in healthy women. Meanwhile, IgM was detected in 23% of patients.

The anti-inflammatory therapy was received by all the examined patients in accordance with the identified pathogens. An important stage was the combination of anti-inflammatory drugs with interferon. For this purpose we used Laferobion - human IFN dosage form, obtained by means genetic engineering methods from E. coli culture, which gene apparatus a plasmid (DNA portion), containing human IFN gene, was integrated in. It provides comprehensive antiviral and immunomodulatory effect.

As additional components the following are used in Laferobion manufacture:

*Ascorbic acid* (vitamin C) – it is raising the number of lymphocytes, enhancing neutrophil leukocytes activity, interferon production, stimulating macrophages to potentiate the antitumor immunity. Due to its antioxidant properties the transition of pro-carcinogens into carcinogens is inhibited.

*Alpha-Tocopherol acetate* (Vitamin E) - activates immuno-competent cells, enhances the IFN production, prevents the formation of large immune complexes caused by the damaging factors: its antioxidant effect protects T- and B-lymphocytes from the inhibitory action of free radicals, and as a result it normalizes the immune system’s activity.

After the treatment performed, normalization of the local immunity was observed: the levels of secretory IgA, IgA, lysozyme indicators did not practically differ from those in healthy women (see Figure 2.). The obtained data testify to the presence of the pronounced immunomodulatory action of Laferobion drug (IFN-alpha-2a), which lies in normalization of both systemic and local immunity.

Thus, inclusion of Laferobion into the treatment regimen of patients with PID allows improving the efficiency of traditional therapy and achieving positive results in the treatment.

CONCLUSIONS

1. Chronic inflammatory diseases of internal genital organs are characterized by systemic disorders of the immune homeostasis, wasting of immune cells, decreased production of humoral protection factors.
2. Based on the nature and type of immune disorders identified, it is reasonable to include immunotropic drugs promoting stimulation of nonspecific resistance factors and having immunomodulating properties into the comprehensive anti-inflammatory therapy.

3. Laferobion (BIOPHARMA) has an integral antiviral and immunomodulatory effect, which allows to efficiently use it in the treatment of pelvic inflammatory disease.

**Immune therapy in the comprehensive treatment of the internal genital organs inflammatory diseases**

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**Key words:** inflammatory diseases of the internal genital organs, infertility, local immunity, immune therapy.

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