Original Research Article

Clinical and immunological evaluation of application of Ronkoleukin in nonspecific vulvovaginitisatadolescent girls

Package insert

According to immunological parameters there wasfound that during the sub-acute there is the secondary immune deficiency and the immunodeficiency is absent during the acute.

During the acute phagocytic function of the local secret is satisfactory, and during the sub-acute against satisfactory absorption function there is a decrease of bacterial growth-inhibitory activity of vaginal secretions, indicating the necessity of correction of phagocytic component of these patients.

Our research has shown that the inclusion of Ronkoleukin in the complex therapy has clinically significant effects: rapid relief of symptoms of local inflammation, accelerated elimination of the causative pathogen and significantly shortening periods of treatment.

The purpose of the research

Clinical and immunological evaluation of Ronkoleukin using depending on the routes of entry in the treatment of non-specific vulvovaginitis among adolescent girls in different variants of the disease state.

Design of there search

Prospective study

Methodology

From 2006 to 2010 years by the assignment of adolescent therapist and upon periodic screening the adolescent girls with a variety of complains for genitalia were examined in the child and adolescent consulting room in Municipal Polyclinic № 11 and “The City Center of Human Reproduction” in Almaty.

Microbiological and immunological studies were performed in the hospitals of Almaty: "The Scientific Center for Obstetrics, Gynecology and Perinatology”, “The Scientific Center of Pediatrics and Pediatric Surgery” of the Ministry of Health of the Republic of Kazakhstan, “The City Center of Human Reproduction”, Municipal Polyclinic № 11, ”The City Skin and Venereal Diseases Dispensary of Almaty”.

1. Analysis of anamnestic data of complaints and clinical symptoms

2. Microbiological analysis - the bacterioscopic and bacteriological examination of the discharge from urethra, vagina and vestibule of vagina

3. Enzyme immunoassay for sexually transmitted diseases to exclude specific pathogens of

vulvovaginitis

4. Immunoassay

The quantitative study of the main lymphocyte populations and subpopulations was performed with the help of double label with monoclonal antibodies on the laser flow cytometry manufactured by “Status” (LLC "Sorbent"/ Podolsk, Russia). Analysis of the samples was carried out on a flow cytometer FacsCalibur (Becton Dickenson) in the Cell Quest program

The next panel was used in the study of antibodies:

• CD45/CD14 (to identify the lymphocyte population and discharge of lymphocytes in low-angle light scattering and SSC);

• IgG1/IgG2 (to control non-specific binding of lymphocytes with anti-human antibodies and lymphocytic discharge with negative fluorescence);

• CD3/CD19 (for determination of contained thymus-processed lymphocytes and bursal lymphocyte respectively);

• CD4/CD8 (to determine the number of t-helper cells and cytotoxic t-lymphocytes);

• CD3/HLA-DR (to determine the number of thymus-processed lymphocytes);

• CD3/CD16+56+ (to determine the natural killers).

5. Cytokine study

The relevant enzyme immunoassay test system, manufactured by LLP "Cytokine" (St. Petersburg/Russia) we used to determine IL-6.

To define IFN-γ and TNF-α the relevant test-systems for enzyme immunoassay of IFN-γ – EIA-BEST and TNF-α - EIA-BEST were used, manufactured by CJSC "Vector-Best” (Koltsovo, Novosibirsk region of Russian Federation).

6. Evaluation of neutrophil phagocytic rate of vaginal discharge

7. Trans-abdominal ultrasound was held by using "ALOKA SSD-500"

124 menstruate adolescent girls from 11 to 18 years, not sexually active, were selected to accomplish the target objective.

I group -20 healthy patients (control)

II group - 62 patients with per-acute nonspecific vulvovaginitis

III group - 42 patients with sub-acute nonspecific vulvovaginitis

The selection criteria in the II and III groups were the lack of systemic and local antimicrobial therapy during one month before this examination, clinical and microbiological confirmation of the diagnosis, excluded sexually transmitted diseases, vaginal foreign body, complaints, consent of patients’ mothers for research and treatment.

In cooperation with the scientific consultant and immunologist, M.D., Professor A. Kurmanova there was developed a dosage schedule of roncoleukine in the complex therapy,

based on the results of the analyses of the immune and cytokine status and phagocytic vaginal.system.

II group - 62 patients with per-acute nonspecific vulvovaginitis

II A– 20 patients with sub-acute nonspecific vulvovaginitis

(Standard therapy + roncoleukine 250 000 U/ml vaginal irrigation once a day).

II B– 25 adolescent girls with sub-acute

(Standard therapy + roncoleukine 250 000 U/ml twice subcutaneously, every other day).

II C – 17 adolescent girls with sub-acute (standard therapy)

III group - 42 patients with acute nonspecific vulvovaginitis

(standard therapy + roncoleukine 250 000 U/ml twice subcutaneously, every other day).

Standard therapy of 7-10 days included (1;2):

- compliance with hygiene rules, restricted diet of digestible carbohydrates and irritating substances, and increase of consumption of fresh fruits and vegetables;

- the local treatment (washing of the vulva, vaginal disinfection processing, application of drugs with anesthetic, astringent desensitizing and/or other effects on the vulva);

- antimicrobials (in compliance with receptiveness of microbial population);

- antimycotics (for prevention of dysbacteriosis);

- decongestant (for the main indication);

- vitamin product;

- biogenic stimulant;

- eubiotics.

The immunomodulatory drug roncoleukine was included into the complex therapy.

The drug Roncoleukin (interleukin-2 human recombinant) has been considered and recommended to the registration by the Pharmacopoeia State Committee of the Republic of Kazakhstan (Protocol No. 92 dated 28/11/2001).

Roncoleukine (recombinant human interleukin) has been considered and recommended to the registration by the Pharmacopoeia State Committee of the Republic of Kazakhstan (Protocol No. 92 dated 28/11/2001).

The drug is produced by LLC “BIOTECH Ltd.”, St Petersburg (3).

Ingredients: 1 ampoule (1 ml) contains recombinant human interleukin - 0.25 mg (250 000 IU) and excipients: sodium lauryl sulfate, mannitol, dithiothreitol, ammonium hydrogen carbonate, water for injection.

Patients’ mothers were informed and obtained their consent to the treatment according to the Law of the Republic of Kazakhstan dated 07.07.2006 №170-3 «National Health Protection Act» Article 31.

Efficacy of the drug was assessed by patient complaints, inspection, and data of microbiological studies of vaginal discharge, immune state, cytokine status, and local phagocytic system on the 7th and 14th days of treatment.

We did not observe any serious side effects, common reactions to the drug administration in the form of anaphylaxis, urticaria; in 2 cases the patients had low-grade pyrexia, which took place independently, and in 3 cases the local reaction to the drug administration was expressed by moderate abnormality that was stopped by applying hot compress.

The statistical processing of the collected material was held by using the licensed software packages StatSoft Statistica 6.0 and Microsoft Excel XP, generally accepted methods of the variation statistics with the calculation of the arithmetic mean (M), the moderate mean (m); the confidence level results were observed within 95% ($p < 0.05$) on the criteria of Student (t) with respect to the medical, biological and mathematical studies. To examine the relationship between measures using the correlation coefficient r.

The weak link was observed when $r \leq 0,3$, moderate - 0,31-0,5, noticeable - 0,51-0,7, strong - > 0.7.

Keywords:

Nonspecific vulvovaginitis, girl, adolescent, immunity, cytokines, phagocytosis, recombinant human interleukin (roncoleukine).

Introduction

In the structure of gynecological disorders at girls and adolescents vulvovaginitis occupies a leading place (4) .

According to scientific studies, the frequency of vulvovaginitisis up to 93 per cent for girls, for adolescents it goes up to 53%, while 60% has a recurrent character (5;6;7;8;9;10;11;12).

At the present stage there is no doubt that the microbial factor prevails in the pathogenesis of nonspecific vulvovaginitis of the adolescent girls (13;14;15;16;17).

Local protection of female genital organs is due to their anatomical and physiological characteristics, the presence of normal microflora, humoral and cellular factors of immunity.

Common infectious disease accompanied by decrease in immunity, as well as the hormonal diseasebreak the qualitative and quantitative composition of the vaginalmicroflora that facilitates the invasion of pathogenic microorganisms and can lead to the development of inflammatory processes caused by opportunistic pathogenic bacterium (18).

The opportunistic pathogens, involved in the inflammatory process, do not contain highly toxic poisons, but they are dangerous for hypernormal promoting of inflammation mediators of micronychia microorganism.

The practical significance of vulvovaginitis is defined by the fact that they lead to the formation of synechia of the labia, genital infection, disruption of menstrual function, which can cause serious disorders of reproductive function in the future (19;20).

The necessity of the clinical relevance of the study of the immunological aspects of vulvovaginitis is that each immune imbalance increases the probability of the progression of the disease, favours the development of complications, the allergization of the organism and the chronization of the process.

The evaluation of the general and of the local immunity at vulvovaginitis is one of the challenging issues, taking into account the essential role of the immune-pathological mechanisms at the chronization and at the retrocessions of the process.

Opportunistic microorganisms which take part in an inflammatory process do not contain any high-toxic poisons, but they are dangerous due to their excessive activation of the inflammatory mediators of the microorganism.

At present time the diagnostic significance of the evaluation of the cytokines concentration level consists in the statement of the very fact of its increasing or reducing during a concrete disease, at that it is appropriate to detect the concentration of pro-inflammatory as well as of anti-inflammatory cytokines in the dynamic of the disease development to be able to evaluate the severity and to make prognosis of the course of disease (21;22;23).

In this connection the search of the new immunological drugs in the complex therapy of vulvovaginitis allowed to examine the use of Interleukin-2 human recombinant (Ronkoleukin) in the complex therapy of nonspecific vulvovaginitis at adolescent girls with different kinds of diseases and introduction of the drug.

For the diagnosis and treatment of vulvovaginitisit is not possible to rely solely only on a visual assessment of the genitalia and the discharge from the vagina;it is also necessary to provide the microbiological research and study of the immune status, local cytokine, andphagocytic system.

165
166 Table 1

167 The main complaints and clinical manifestations of nonspecific vulvovaginitis adolescent girls (M ± m)

Indices	Group I	Group II A	Group II B	Group II C	Group III
Complaints					
Discharge	-	3(15,0±3,8)	6(24,0±4,7)	7(41,2±3,9)	19 (45,2±6,0)
Redness	-	11(55,0±7,0)	15(60,0±7,1)	11(64,7±4,8)	30 (71,4±7,1)
Vulvovaginal pruritus	-	7(35,0±5,7)	12(48,0±6,5)	12(70,6±5,0)	24 (57,1±6,6)
Heat	-	3(15,0±3,8)	11(44,0±6,3)	11(64,7±4,8)	22 (52,4±6,4)
Vulvovaginal pain	-	3(15,0±3,8)	8(32,0±5,4)	10(58,8±4,6)	18 (42,9±5,9)
Painfulurination	-	1(5,0±2,2)	3(12,0±3,4)	2(11,8±2,2)	5 (11,9±3,4)
Examination					
Hyperaemia of the labia majoria	-	6(30,0±5,3)	7(28,0±5,1)	5(29,4±3,4)	23(54,8±6,5)
Hyperaemia of the vestibule of vagina	-	11(55,0±7,0)	22(88,0±8,3)	17(100,0±5,8)	39 (92,9±7,5)
Hyperaemiaofthe urethral meatus	-	13(65,0±7,5)	9(36,0±5,7)	7(41,2±3,9)	16 (38,1±5,7)
Oedemataofthelabia majoria	-	4(20,0±4,4)	4(16,0±3,9)	4(23,5±3,0)	13(30,9±5,2)
Oedemataof the vestibular mucous membrane	-	8(40,0±6,1)	11(44,0±6,3)	11(64,7±4,8)	30 (71,4±7,1)
Oedemataoftheurethral meatus	-	4(20,0±4,4)	6(24,0±4,7)	5(29,4±3,4)	11 (26,2±4,8)
Hyperaemiaofthe perineum	-	6(30,0±5,3)	8(32,0±5,4)	6(35,3±3,7)	16 (38,1±5,7)
Pathologicdischargefrom the genital tracts					
The value of discharge					
Low	6(30,0±5,3)	14(70,0±7,8)	18(72,0±7,7)	12(70,6±5,0)	-
Moderate	14(70,0±7,8)	6(30,0±5,3)	5(20,0±4,4)	3(17,6±2,6)	25 (59,5±6,7)
Plethorical	-	-	2(8,0±2,8)	2(11,8±2,2)	17 (40,5±5,8)
Colour of discharge					
Off-white	20(100,0±8,9)	-	-	-	-
Off-white–xanthic	-	13(65,0±7,5)	8(32,0±5,4)	6(35,3±3,7)	8 (19,0±4,2)
Pyromucous (xanthic)	-	7(35,0±5,7)	7(28,0±5,1)	8(47,1±4,2)	17 (40,5±5,8)
Pyogenic (xanthic- greenish)	-	-	10(40,0±6,0)	3(17,6±2,6)	17 (40,5±5,8)

The clinical picture of sub-acute and acute nonspecific vulvovaginitis was presented bythe symptoms of vulvitisand urethritis.

The most common complaints in the acute period were redness in the genital area, amyctic, burning and discharge from the genital tract, and on examination there were frequently identified hyperemia and swelling of the pronaus.

In the sub-acute the main complaints were redness,amyctic, and on examination there was seen hyperemia of theurethral meatus and pronaus.

In the clinical picture in acute course of the disease the moderate discharge from the reproductive tracts was more evident, and the sub-acute discharge waslow.

It drew attention to the fact that the patients in both groups had the discharge of pathological character from the genital tract during several weeks, and only when the amyctic, burning, discomfort had appeared in the genitals,they turned to a specialist.

On examination of the patients there was noticed the dissonance in the intensity of inflammatory symptoms in the sub-acute.

Objectively sub-acute vulvovaginitis was characterized by isolated hyperemia of internal surfaces of the labia and labia majoria, pronausand injected vessels of the vulva, that indicated the long-term inflammation.

The absence of subjective sensations, the limit of inflammation by inflammation of glands and urethral lacunes, hyperemia of stasis and swelling of pronausconfirm that vulvovaginitis is sub-acute in nature.

Table 2

Immune status and phagocytic function of vaginal discharge in the examined before treatment (M ± m)

Indices	Group I healthy patients (n=20)	Group II with sub-acute form (n=62)	Group III with acute form (n=42)
CD3+(%)	64,5 ± 0,3	59,32±1,9*	69,2 ± 6,4
CD4+(%)	53,9 ± 1,0	49,4 ±5,1	53,0 ± 8,1
CD8+(%)	23,2 ± 0,7	20,8 ±2,7	22,0 ± 5,3
CD20+(%)	15,3 ± 0,7	12,4 ± 0,6*	14,9 ± 4,1
ИНФγ (pg/ml)	11,02 ± 0,74	12,49 ± 6,49	17,32 ± 12,2
ФНОα (pg/ml)	4,48 ± 0,57	3,99 ± 0,70	4,14 ± 1,7
ИЛ-6 (pg/ml)	22,62 ± 1,80	38,24 ± 3,22	36,5 ± 26,7
Phagocytic index, idiopathic, %	22,4±5,4	35,8±5,8	47,2±15,2
Phagocytic number, idiopathic	8,0±0,2	5,9±1,4	6,7±1,5
Phagocytic index, induced by pyrogenal, %	36,3±4,7	48,8±8,4	50,0±10,0
Phagocytic number, induced by pyrogenal	10,0±0,3	6,6±1,2*	8,7±0,7
HCT idiopathic, %	24,5±13,0	10,1±4,12	14,2±6,2
HCT stimulated by pyrogenal, %	44,1±5,7	12,4±4,9*	32,3±2,3***

* The disparity is accurate at P≤0.05 between the Groups I and II .
** The disparity is accurate at P≤0.05 between the Groups I and III.
*** The disparity is accurate at P≤0.05 between the Groups II and III.

It was found that the patients with sub-acute disease had a significant downstream of the relative content of Cd3+, indicating the oppression of their differentiation. There was also recorded a significant reduction of Cd20+. With regard to the value of CD4+ and CD8+ compared to the control group there was a tendency to decrease.

Study of immunological parameters with acute disease has shown that there are no reliable differences in comparison with the control group, although there was watched a trend towards downstream of CD4 +, Cd8 + and Cd20 + cells and the trend of increase in Cd3 +, due to the dispersion of individual value.

On the basis of the revealed violations by the parameters of the immune status, it can be assumed that in the sub-acute there is a recurrent immunodeficiency, and there is no deficiency during acute.

In all studied groups the average cytokine profile's value in peripheral blood did not differ significantly from the average value of the control group in connection with a wide range of value.

Therefore, the further analysis of cytokine outputwas conducted by the percentage of occurrence of elevated and high value.

Table 3

The frequency of the increased cytokine concentration in the examined patients(M ± m)

IndicesPg/ml	GroupI	Group II	Group III
γ-Interferon	0	15,0±8,0*	23,8±6,6**
TNF α	10,0±6,7	20,0±8,9	21,4±6,3
IL-6	0	20,0±8,9*	14,3±5,4**

* The disparity is accurate at P≤0.05 between the 1st and the 2nd groups.
** The disparity is accurate at P≤0.05 between the 1st and the 3rd groups.

In the I group the high output of cytokines IFN-γ, TNF-αand IL-6 were not observed. The increased output of IFN-γwas recorded at 10% of examined patients.

In the second group, there was an increase in output of IFN-γat 15% of the patients, with adequately high content(in 4 times), recorded only at one patient (5%. The elevated level of TNF-αoutputwas registered at 20% of the patients;herewith there was no any highest production (in 4 or more times). At the same time there was the increased output of IL-6 at 20%. It should be noted that high (in 5-13 times) output of IL-6 was at 15% (3/20). When comparing the rates of occurrence of high content with I group, the increased output of IFN-γ and IL-6 was more often

registered in the 2nd group. However, adequate cytokine explosion with the activation of decreasing immunity was observed only in 1/6 cases.

In the III group the enhancement of IFN-γ output was observed at 23.8% of the surveyed. Herewith, the adequately high content (in 4 times) was recorded at 3/42 (7.1%) of the surveyed. An elevated output of TNF-α was at 21.4% of surveyed, with the highest (in 4 or more times) was not a product of one. The increased production of IL-6 was observed at 14.3% of the patients, while high production was recorded at 4.7% of the patients. In comparing values of occurrence of high content with group I, increased production of IFN-γ and IL-6 was significantly recorded in group III ($p \leq 0.05$).

Consequently, nearly 1.6 times more girls and adolescents with acute nonspecific vulvovaginitis have the increased output of proinflammatory cytokine IFN-γ than with sub-acute, indicating the direct dependence of the activity level of proinflammatory cytokine from the clinical course of the inflammatory process. Although, itshigh output does not exist in both variants of the disease.

The data obtained give the evidence of the oppression of anticontagious immunity both cellular and humoral, as TNF-α has the co-stimulant function for T-cell activation and activationof mononuclear phagocytes, also it helps the antibody formation by B-cells, and IL-6 is responsible for the specificity and the adequacy of immune reactions. This fact is due, first of all, with the presence of different pathological changes of immune system.

In the group I the absorptive function of the vaginal discharge complies with the similar parameters of healthy women of reproductive age, while digestible function of the vaginal discharge is more evident in adolescence.

The phagocytic function of vaginal discharge at the adolescent girls of the II group in comparison with I group was observedin some improvement of the average absorption capacity of vaginal discharge, but did not differ significantly. Herewith, the digestible ability of the vaginal discharge was decreased, resulting in decreasing the NBTR-test value in the stimulated version (NBTR-test stim. - $12.4 \pm 4.9\%$, $p \leq 0.05$). The spontaneous value of NBTR-test was below the equivalent control values, but not significantly different, due to the wide scatter in values.

In the III group all values both the absorption and digestive functions of vaginal discharge did not differ significantly from those in the Igroup, however, there has been a tendency to increase of the valueboth spontaneous and stimulated phagocyte index, and to the downstream of the phagocyte number

By comparison of the sub-acute value there was no substantial difference in absorption capacity, but was noted a significant reduction of bactericidal activity in sub-acute in comparison with acute.

Thus, in the acute with nonspecific vulvovaginitis at young girls and adolescents thephagocytic function of the local dischargeis satisfactory; and during the sub-acute against satisfactory absorption function thereisobservedthe decrease of bactericidal activity of the vaginal discharge, which demonstrates the need for the phagocyte correction of these patients.

Table 4

Dynamic of complaints and of clinical signs in theGroupIIA (M ± m)

Analysed indices	until the treatment	on the 7th day	on the 14th day
Complaints			
Discharge	3(15.0±3.8%)	7(35±5.7%)*	3(15±3.8%)
Redness	11(55.0±7.0%)	8(40±6.1%)*	2(10±3.1%)
Vulvovaginal pruritus	7(35.0±5.7%)	5(25±4.9%)	2(10±3.1%)
Heat	3(15.0±3.8%)	1(5±2.2%)*	-
Vulvovaginal pain	3(15.0±3.8%)	2(10±3.1%)	-
Painfulurination	1(5.0±2.2%)	-*	-
Examination			
Hyperaemia of the labia majoria	6(30.0±5.3%)	3(15±3.8%)*	1(5±2.2%)**
Hyperaemia of the vestibule of vagina	11(55.0±7.0%)	5(25±4.9%)*	3(15±3.8%)
Hyperaemiaofthe urethral meatus	13(65.0±7.5%)	-*	-
Oedemataofthelabia majoria	4(20.0,0±4.4%)	-*	-
Oedemataof the vestibular mucous membrane	8(40.0±6.1%)	2(10±3.1%)*	-
Oedemataoftheurethral meatus	4(20.0±4.4%)	-*	-
Hyperaemiaofthe perineum	6(30.0±5.3%)	3(15±3.8%)*	2(10±3.1%)
Pathologicdischargefrom the genital tracts			
The value of discharge	14(70.0±7.8%)	11(55±7.0%)	5(25±4.9%)
Low	6(30.0±5.3%)	9(45±6.4%)*	15(75±8.0%)
Moderate	-	-	-
Plethorical			
Colour of discharge	-	7(35±5.7%)*	11(55±7.0%)
Off-white	13(65.0±7.5%)	9(45±6.4%)*	9(45±6.4%)
Off-white–xanthic	7(35.0±5.7%)	4(20±4.4%)*	-
Pyromucous (xanthic)	-	-	-

* The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 7th day

** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day

291 Table 5

292 Dynamic of the immunological indices and of the indices of the local phagocytic system, Group II A(M ± m)

Indices	Control group (n=20)	Group IIA		
		until the treatment	on the 7th day	on the 14th day
CD3+ (%)	64.5 ± 0.3	59.32±1.9*	61.3 ± 1.6**	64.6 ± 1.4 #
CD4+ (%)	53.9 ± 1.0	49.4 ±5.1	49.6 ±1.6	49.5 ±2.0
CD8+ (%)	23.2 ± 0.7	20.8 ±2.7	21.4 ±1.2	21.6 ±1.1
CD20+ (%)	15.3 ± 0.7	12.4 ± 0.6*	13.2 ± 1.1	13.6 ±0.86
IL-6 (pg/ml)	22.6 ± 1.8	38.2±3.22	23.9 ± 0.87	22.3 ± 1.3
Interferon (pg/ml)	11.02 ± 0.74	12.5 ±6.5	12.04 ±0.37	12.1 ±0.23
TNF (pg/ml)	4.48 ± 0.6	3.9 ±0.7	4.57±0.36	4.4 ±0.2
* The disparity is accurate at P≤0.05 between the examination and until treatment ** The disparity is accurate at P≤0.05 between the examination and on the 7th day *** The disparity is accurate at P≤0.05 between the examination and on the 14th day # The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day				
Phagocytic index, idiopathic, %	22.4±5.4	35.8±5.8	-	60.2±11.4**
Phagocytic number, idiopathic	8.0±0.2	5.9±1.4	-	7.9±1.6***
Phagocytic index, induced by pyrogenal, %	36.3±4.7	48.8±8.4	-	65.8±9.8**,***
Phagocytic number, induced by pyrogenal	10.0±0.3	6.6±1.2*	-	9.8±0.7***
Phagocytic index, induced by ronkoleukine, %	44.5±4.7	92.6±3.3*	-	99.3±0.4**
Phagocytic number, induced by ronkoleukine	10.0±0.3	14.0±1.9*	-	16.3±1.07**
HCT idiopathic, %	24.5±13.0	10.1±4.12	-	20.0±2.7
HCT stimulated by pyrogenal, %	44.1±5.7	12.4±4.9*	-	22.3±6.2**
HCT stimulated by ronkoleukine, %	44.1±5.7	35.5±7.25	-	52.0±4.6
* The disparity is accurate at P≤0.05 between the control and until the therapy. ** The disparity is accurate at P≤0.05 between the control and after the therapy. *** The disparity is accurate at P≤0.05 between until and after the therapy.				

Before the treatment in cytokine composition the averages did not differ significantly from the control group average.

The same pattern for mean values was observed on the $7^{th}$and 14^{th}days after treatment. But when comparing the percentage of occurrence of high levels before treatment there was observed the high output of IFN-γat 15% of the patients, output of TNF-α and IL-6 - at 20%, of the patients, output of TNF-αat 25% of the patientsincreasedon the 7^{th}day after treatment, and increasing output of cytokines was observedon the 14^{th}day. These changes may indicate an inflammatory process remitting, preceded by activation of exogenous cytokine.

Before the treatment in the group II (A) in comparison with the group I the average absorption capacity of vaginal discharge was not different; the digestion ability of vaginal discharge was reduced, that resulted in reduction of the NBTR-test value in the Pirogenalstimulated version. In loading tests with Ronkoleukin in vitro, there was a sharp increase of phagocyte index, phagocyte number and NBTR-test,which indicated a positive response of vaginal mucus.

Through 7 days after the vaginal irrigation by Ronkoleukin a local secret reaction was the following: the value of spontaneous and induced by Pirogenalthe phagocyte index, phagocyte number, and NBTR-testsharply increased.

In comparison with the individual changes, it was observed a significant increase in spontaneous phagocytic number to 139.2%, phagocyte number induced by Ronkoleukinto 113.5%, spontaneous NBTR-test, induced by Pirogenal and Ronkoleukinto 353.3%, 220 percent and 161.9% respectively.

Thus, in the sub-acute the local application of Roncoleukinleads to the increased absorption and oxygen-dependent bactericidal ability of vaginal secretion, which contributes to the rapid involution of clinical aspects.

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317 Table 6

318 Dynamic of complaints and of clinical signs in theGroupIIB(M ± m)

Analysed indices	until the treatment	on the 7th day	on the 14th day
Complaints			
Discharge	12(48.0±6.5%)	7(28.0±5.1%)*	2(8.0±2.8%)**
Redness	19(76.0±7.8%)	8(32.0±5.4%)*	-**
Vulvovaginal pruritus	12(48.0±6.5%)	4(16.0±3.9%)*	2(8.0±2.8%)**
Heat	11(44.0±6.3%)	-*	-**
Vulvovaginal pain	8(32.0±5.4%)	-*	-**
Painfulurination	3(12.0±3.4%)	-*	-**
Examination			
Hyperaemia of the labia majoria	14(56.0±6.9%)	4(16.0±3.9%)*	1(4.0±2.0%)**
Hyperaemia of the vestibule of vagina	22(88.0±8.3%)	9(36.0±5.7%)*	-**
Hyperaemiaofthe urethral meatus	9(36.0±5.7%)	-*	-**
Oedemataofthelabia majoria	8(32.0±5.4%)	-*	-**
Oedemataof the vestibular mucous membrane	17(68.0±7.5%)	2(8.0±2.8%)*	-**
Oedemataoftheurethral meatus	6(24.0±4.7%)	-*	-**
Hyperaemiaofthe perineum	11(44.0±6.3%)	2(8.0±2.8%)*	2(8.0±2.8%)**
Pathologicdischargefrom the genital tracts			
The value of discharge	-	18(72.0±7.8%)*	9(36.0±5.7%)**
Low	14(56.0±6.9%)	7(28.0±5.1%)*	16(64.0±7.3%)**
Moderate	11(44,0±6,3%)	-*	-**
Plethorical			
Colour of discharge	-	6(24.0±4.7%)*	14(56.0±6.9%)
Off-white	8(32.0±5.4%)	11(44.0±6.3%)	11(44.0±6.3%)**
Off-white–xanthic	7(28.0±5.1%)	8(32.0±5.4%)	-**
Pyromucous (xanthic)	10(40.0±6.0%)	-*	-**

* The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 7th day
** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day

319 Table 7

320 Dynamic of the lymphocytes subpopulations rates in the setting of the treatment, the Group II B(M ± m)

Indices	Group I A	Group II B		
		until the treatment		until the treatment
CD3+ (%)	64,5 ± 0,3	59,32±1,9*	61,3 ± 1,6**	64,6 ± 1,4 #
CD4+ (%)	53,9 ± 1,0	49,4 ±5,1	49,6 ±1,6	49,5 ±2,0
CD8+ (%)	23,2 ± 0,7	20,8 ±2,7	21,4 ±1,2	21,6 ±1,1
CD20+ (%)	15,3 ± 0,7	12,4 ± 0,6*	13,2 ± 1,1	13,6 ±0,86
IL-6 (pg/ml)	22,6 ± 1,8	20,75±1,54	24,17±0,62	20,64±1,54
Interferon (pg/ml)	11,02 ± 0,74	10,44±0,57	11,50±0,28	12,01±0,23
TNF (pg/ml)	4,48 ± 0,6	4,88±0,29	5,07±0,22	4,64±0,20

* The disparity is accurate at P≤0.05 between the examination and until treatment
** The disparity is accurate at P≤0.05 between the examination and on the 7th day
# The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day

Before treatment in the group II B there was record of decrease of CD3 + and CD20 + lymphocytes in the valueof lymphocyte subpopulation composition.On the 7thday rate of Cd3 + increased slightly, but continued to be significantly reduced, and the content of Cd20 + turned up to stated value.

An increase of CD3+lymphocytes to the level of stated value was recorded on the 14th day, and by comparing value before and after the treatment there was registered a significant improvement of this indicator.

Thus, the appointment of Roncoleukin subcutaneously twice alternate days in the sub-acute leads to the normalization of mainvalue of lymphocyte subpopulation composition.

Table 8

Dynamic of complaints and of clinical signs in theGroupII C(M ± m)

Analysed indices	until the treatment	on the 7th day	on the 14th day
Complaints			
Discharge	7(41,2±3,9)	7(41,1±6,1)	-**
Redness	11(64,7±4,8)	3(17,6±2,6)*	-**
Vulvovaginal pruritus	12(70,5±5,0)	4(23,5±3,0)*	1 (5,9±1,5)**
Heat	11(64,7±4,8)	-*	-**
Vulvovaginal pain	10(58,8±4,6)	-*	-**
Painfulurination	2(11,8±2,2)	-*	-**
Examination			
Hyperaemia of the labia majoria	9(52,9±4,4)	-*	-**
Hyperaemia of the vestibule of vagina	17(100±5,8)	2(11,7±3,3)*	-**
Hyperaemiaofthe urethral meatus	7(41,2±3,9)	-*	-**
Oedemataofthelabia majoria	5(29,4±3,4)	-*	-**
Oedemataof the vestibular mucous membrane	13(76,5±5,2)	-*	-**
Oedemataoftheurethral meatus	5(29,4±3,4)	-*	-**
Hyperaemiaofthe perineum	5(29,4±3,4)	2(11,8±2,2)*	-**
Pathologicdischargefrom the genital tracts			
The value of discharge	-	9(52,9±4,4)*	7(41,2±3,9)**
Low	11(64,7±4,8)	8(47,1±4,2)*	10(58,8±4,6)
Moderate	6(35,3±3,7)	-*	-**
Plethorical			
Colour of discharge	-	7(41,2±3,9)*	15(88,2±5,5)**
Off-white	-	7(41,2±3,9)*	2(11,2±2,2)**
Off-white–xanthic	10(58,8±4,6)	3(17,6±2,6)*	-**
Pyromucous (xanthic)	7(41,2±3,9)	-*	-**

* The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 7th day

** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day

Table 9

Dynamic of the immunological indices, Group II C(M ± m)

Indices	Group I	Group II C		
		until the treatment	on the 7th day	on the 14th day
CD3+(%)	64,5 ± 0,29	63,19±0,80	62.48±0,94	66,25±0,71
CD4+(%)	53,95 ± 0,9	47.13±2,43	50.25±1,25	46,18±1,06
CD8+(%)	23,2 ± 0,7	19.94±1.00	22.78±0,84	24,83±1,45
CD20+(%)	15,3 ± 0,7	11,52±0,55	16.61±1,04	16.06±0,79
IL-6 (pg/ml)	22,6 ± 1,8	19,77±1,46	26,25±0,65	24,22±0,49
Interferon(pg/ml)	11,02 ± 0,74	10.35±0,45	12.02±0,33	12.68±0,71
TNF(pg/ml)	4,48 ± 0,57	4.32±0,30	5.55±0,13	3.95±0,15

Table 10

Dynamic of complaints and of clinical signs in theGroupIII(M ± m)

Analysed indices	until the treatment	on the 7th day	on the 14th day
Complaints			
Discharge	19(45.2±6.1%)	14(33.3±5.4%)	2(4.8±2.2%)**
Redness	30(71.4±7.1%)	11(26.2±4.8%)*	-**
Vulvovaginal pruritus	24(57.1±6.6%)	8(19.0±4.2%)*	3(7.1±2.6%)**
Heat	22(52.4±6.4%)	-*	-**
Vulvovaginal pain	18(42.9±5.9%)	-*	-**
Painfulurination	5 (11.9±3.4%)	-*	-**
Examination			
Hyperaemia of the labia majoria	23(54.8±6.5%)	4(9.5±3.0%)*	1(2.4±1.5%)**
Hyperaemia of the vestibule of vagina	39(92.9±7.5%)	11(26.2±4.8%)*	-**
Hyperaemiaofthe urethral meatus	16(38.1±5.7%)	-*	-**

Oedemataofthelabia majoria	13(31.0±5.3%)	-*	-**
Oedemataof the vestibular mucous membrane	30(71.4±7.1%)	2(4.8±2.2%)*	-**
Oedemataoftheurethral meatus	11(26.2±4.8%)	-*	-**
Hyperaemiaofthe perineum	16(38.1±5.7%)	4 (49.5±3.0%)*	2 (4.8±2.2%)**
Pathologicdischargefrom the genital tracts			
The value of discharge	-	27 (64.3±6.9%)*	16(38.1±5.7%)**
Low	25(59.5±6.7%)	15((35.7±5.5%)*	26(61.9±6.8%)
Moderate	17(40.5±5.8%)	-	-**
Plethorical			
Colour of discharge	-	13(31.0±5.2%)*	29(69.0±7.0%)**
Off-white	8 (19.0±4.2%)	18 (42.9±5.9%)*	13(31.0±5.2%)**
Off-white–xanthic	17(40.5±5.8%)	11 (26.2±4.8%)	-
Pyromucous (xanthic)	17(40.5±5.8%)	-*	-**
* The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 7th day ** The disparity is accurate at P≤0.05 between the beginning of the treatment and on the 14th day			

Table 11

Dynamic of the immunological indices, Group III(M ± m)

Indices	Group I	Group III		
		until treatment	on the 7th day	on the 14th day
CD3+(%)	64,5 ± 0,29	69,2 ± 6,4	62,5 ± 1,6	66,8 ± 1,1
CD4+(%)	53,95 ± 0,9	53,0 ± 8,1	50,6 ±1,6	49,1 ±2,7
CD8+(%)	23,2 ± 0,7	22,0 ± 5,3	22,3 ±1,1	24,04 ±1,2
CD20+(%)	15,3 ± 0,7	14,9 ± 4,1	14,6 ± 1,9	15,3 ±0,8
IL-6 (pg/ml)	22,6 ± 1,8	36,5 ± 26,7	25,3 ± 1,1	22,5 ± 1,9
Interferon(pg/ml)	11,02 ± 0,74	17,32 ± 12,2	11,8 ±0,4	12,3 ±0,5
TNF(pg/ml)	4,48 ± 0,57	4,14 ± 1,7	5,3±0,28	4,3 ±0,4

On the 7th and 14th daysthe value of lymphocyte subpopulation composition did not undergo any significant changes, but the dynamics tended to decrease (normalization) of CD3+ lymphocytes.

Thus, in the acute period twice alternate days subcutaneous injection of Roncoleukin has no effect on the normal values of the immune status, i.e. it has a modulatory effect depending on the initial status.

On the 7thday of the treatment there were no any complaints to dysuria and objectively there were no swelling and hyperemia of external urethral openingand swelling of the labia majoria.

The profuse discharge were completely absent on the 7thtreatment day, corresponding to the data of objective examination.

According to the patient's words there wasan increased vaginal discharge, but the nature of this discharge has changed to ordinary fluor albuswith a gradual transition to the normal vaginal discharge to the 10thday of the treatment.

Thus, the major clinical criteria of inflammatory process remitting were the quantity reduction of the pathologic discharge from the reproductive tract, appearance of light whitish discharge in moderate and scarce quantities, disappearance of vulvovaginal pain, burning sensation in the genitals, decurrence of dysuric syndrome, the blushed mucous membranes of the vagina with an absence of any pathological changes

Conclusion

Thus, in the subacute the appointment of twice alternate days subcutaneous injection of Roncoleukin leads to the normalization of main value of lymphocyte subpopulation.

In the acute Ronkoleukin has no influence on the normal values of the immune status, i.e. it has an effective immune protection depending on the initial status.

Prior to treatment with cytokin composition the mean value did not differ significantly from the control group valuein the sub-acute.

The high percentage of IFN-γvalue before treatment was observed at 15% of the patients, TNF and IL-6 (at 20%, after treatment for 7thday it was reported an increased production of TNF-at 25% of patients, and on the 14thday the increased production of cytokines were not observed. These changes may indicate a decrement of inflammation, which was preceded by the activation of cytokine.

In the acute period prior to treatment inthe cytokine compositionthe value did not differ significantly from the average of the control group.

Interest occurrence of increased production value IFN-γ was observed at 23.8% of patients, TNF - at 21.4% of patients, IL-6 - at 14.3% of patients, which was significantly more frequently than in the control group.

On the 7thday there was evaluated an increased production of IL-6 at 14.2% of the patients, TNF at 78.6%, and on the 14thday the increasing of IL-6 was only 4.8%. In this case the introduction of Roncoleukin has also helped to reduce the inflammatory response.

Changes ofphagocytic activity of the local secretion were observed inthe sub-acute.

After the local application of Ronkoleukin through 7 days reaction of the local secret sharply increased performance spontaneous and induced by pirogenalof phagocytic index, phagocytic number, NBTR-test.

Thus, locally administering of preparation greatly increases the efficiency of patients' treatment with a positive effect on the clinical course of the disease, providing immunomodulatory effects.

All patients with the sub-acute and acute vulvovaginitis should be made an assessment,in addition to clinical examination,for the state of the immune and cytokine status, as well as phagocytic system of vaginal contents in order to address the issue of immune system correction.

Immune system correction by Ronkoleukin locally can be recommended to patients with sub-acute disease.

Application of Ronkoleukin can reduce treatment costs and shorten the time of treatment.

These phenomena were leveled out independently at the 2nd day and were not accompanied by abnormality of general well-being.

In view of the high tolerability and rare complications,the local application can be performed on an outpatient basis.

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