Efficiency of Anaferon in Complex Therapy of Genital Herpes

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We studied clinical efficiency and IFN-inducing activity of anaferon in chronic recurrent genital herpes with high incidence of relapses. The use of anaferon in complex therapy reduced the duration of intoxication symptoms and local symptoms, shortened the duration of the relapse, activated expression of IFN-γ mRNA, and improved IFN-γ-producing capacity of blood leukocytes.

Key Words: *genital herpes; interferon inductors; anaferon*

Genital herpes is a widespread form of herpes-virus infection and a prevalent disease among sexually-transmitted infections. Annually, about 20% patients with newly acquired genital herpes are detected among sexually active population. Herpes-virus infection can be a cause of reproductive disorders, pregnancy miscarriage, premature labor, and fetal pathologies [3,6,7].

Therapy of genital herpes is very difficult because of impossibility of eliminating the virus from the organism, on the one hand, and formation of an immunodeficient state, on the other. The process of virus replication is accompanied by the production of proteins suppressing the immune response [10,14]. Suppression of the reactions of cellular immunity, reduced activity of natural defense factors, impaired IFN-α- and IFN-γ-producing capacity of leukocytes, and dysfunction of the cytokine network were reported [2,5,10]. Therapy of patients with genital herpes is aimed at reduction of the incidence of relapses, their severity and duration and at prevention of virus transmission to sexual partners and to newborn [4,6,9].

According to international standards, acyclic nucleosides are preparations of choice for the treatment of herpes-virus infections. The effect of these preparations consists in inhibition of virus-specific thymidine kinase responsible for amplification of herpes-virus DNA [4,11,12]. However, despite selective antiviral activity of acyclic nucleosides, monotherapy with these drugs is insufficient, because they affect only actively replicating viruses and cannot prevent the relapses after termination of treatment. Immunotherapy as a method of prophylaxis of herpes-virus infection relapses is pathogenetically substantiated in these cases [4,5,12,13]. In light of this, treatment of these patients should be complex and should include the methods of chemo- and immunotherapy.

A modern and promising trend in the treatment and prophylactics of herpes-virus-induced pathologies is the use of preparations modulating the response of the immune system against viral infections [1]. Comprehensive studies showed that antiviral effects of these preparations are mediated by induction of not only IFN, but also other pro- and antiinflammatory cytokines. The synthesis of cytokines IFN-α, IFN-γ, IL-1, IL-12, and TNF-α in herpes simplex infection leads to activation of the major antigen-presenting cells and development of Th1 cell-mediated antiviral immune response.

Anaferon, a representative of these new preparations developed in Russia, contains ultralow doses of antibodies to IFN-γ. The effect of anaferon is based on induction of endogenous IFN-γ, IFN-α, IFN-β and modulation of the expression of functionally coupled cytokines, including IL-2, IL-4, and IL-10 [8]. It was experimentally proven that ultralow doses of antibo-

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dies to IFN-γ can stimulate the reactions of both cellular and humoral immunity via induction of cytokines, which allowed using this preparation in acute and chronic infections caused by herpes simplex virus.

Here we evaluated clinical efficiency and IFN-inducing activity of anaferon as a component of combined therapy in patients with frequently relapsing genital herpes.

MATERIALS AND METHODS

We examined 72 patients (21 men and 51 women) with chronic frequently relapsing herpes-virus infection (CHVI) with genital localization. The majority of patients had more than 5-year history of CHVI. By the moment of examination, the patients reported the appearance of genital lesions more than 8 times per year, some patients up to 2 times per month. The examinees were randomly divided into 3 groups (24 patients per group): group 1 patients received anaferon monotherapy for 21 days; group 2 patients received complex therapy with anaferon for 21 day and acyclovir for 7 days. Patients of group 3 (control) received standard antiviral therapy (acyclovir) during the relapse. Anaferon was given in lingual tablets according to the therapeutic scheme: starting dose 8 tablets per day with subsequent reduction to 3 tablets per day over 3 weeks. Acyclovir was administered in a dose of 200 mg 5 times a day over 7 days.

Therapeutic efficiency was evaluated during exacerbation of CHVI (≤48 h from the relapse onset). By the moment of examination, all patients had clinical signs of genital herpes relapses in the form of vesicular or erosive elements. In men, these elements were located on the penile shaft and glans and in women the lesions were found on large and small pudendal lips and in pubic and gluteal regions.

Clinical efficiency of the complex therapy was evaluated by the time of complete convalescence (complete reepithelization), time of crust formation, and duration of local symptoms (itch, pain, burning at the site of lesion). In parallel, the dynamics of general intoxication symptoms (headache, fever, myalgia, chill, weakness) was evaluated.

For studying the effect of therapy on IFN-synthesizing capacity of blood leukocytes, parameters of the IFN status were determined in dynamics: before (day 1 of exacerbation of genital herpes) and on days 8 and 21 of treatment. IFN-status included the following parameters: level of circulating IFN (serum IFN), spontaneous IFN production; IFN-α production by leukocytes after *ex vivo* induction with viral inductors (Newcastle disease virus, NDV), IFN-γ production after *ex vivo* induction with mitogens (phytohemagglutinin, PGA). Expression of IFN-γ mRNA in peripheral blood mononuclears was evaluated by reverse transcription—PCR.

RESULTS

The distribution of male and female patients in the groups was similar: men and women comprised 25.0-33.3% and 66.7-75.0% of the total number of examinees (Table 1).

Analysis of clinical symptoms and their severity showed that more than half of patients in all groups felt worse during the relapse, about one-third of patients had elevated body temperature (37.0-37.2°C) and headache, about 50% patients complained of pains along the involved nerves. The relapses were often accompanied by mood changes (>80%): irritability, tearfulness, and neurotic disorders were noted.

In group 2 patients, general condition more rapidly improved, the periods of elevated body temperature and headache were shorter than in group 3 patients. The positive effect of anaferon on general condition manifested in shortened duration of fever (by 35%) and of headache (by 15%). Mood disorders (irritability, apathy) persisted for the same time in all three groups (4.5-5.0 days).

During treatment, local symptoms most rapidly disappeared in group 2 patients. The duration of itch and burning decreased by on average 0.6 and 0.9 days, respectively ($p<0.05$), compared to group 3 patients. In group 1 patients, itch, pain, and tingling at the site of lesions disappeared more slowly ($p<0.05$) than in group 3.

In group 2 patients, the period until crust formation was about 3 days and significantly ($p<0.05$) dif-

TABLE 1. Characteristics of Groups of Patients with Recurrent Genital Herpes (*M*±*m*)

Group	Mean age	Mean duration of the disease, years	Men		Women	
			n	%	*n*	%
Group 1	37.1±2.9	10.1±1.9	7	29.2	17	70.8
Group 2	39.4±2.3	9.5±1.6	8	33.3	16	66.7
Group 3	33.7±2.0	7.1±1.1	6	25.0	18	75.0

TABLE 2. Disappearance of Local Symptoms and Terms of Reepithelization against the Background of Therapy (*M±m*)

Symptoms	Group 1	Group 2	Group 3
Itch	5.7±0.3*	3.4±0.2+*	4.0±0.2
Pain	5.5±0.2*	3.2±0.2+	3.5±0.1
Burning sensation	4.5±0.4	2.9±0.3+*	3.8±0.2
Tingling	4.3±0.3*	2.8±0.1+	3.2±0.2
Time of crust formation, days	5.48±0.31*	3.02±0.23+*	3.68±0.22
Time to complete reepithelization, days	6.78±0.32*	5.37±0.38+	5.50±0.32

Note. $p<0.05$ compared to: +group 1, *group 3.

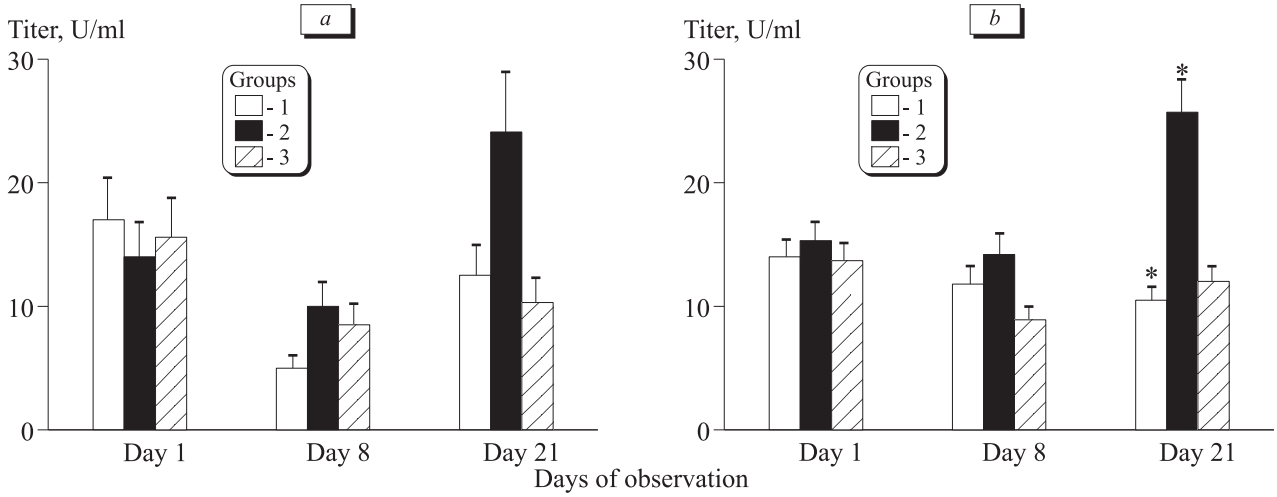

Fig. 1. Dynamics of the level of induced IFN-α (*a*) and IFN-γ (*b*) production during treatment. Here and on Figs. 2, 3: *$p<0.05$ compared to day 1.

fered from the corresponding parameter in groups 1 and 3. In group 1 patients this period was longer and significantly differed from that in group 3.

Evaluation of the IFN status parameters in patients with genital herpes during treatment in different groups revealed some changes in the IFN system. Serum IFN content was not elevated in patients of all groups. After treatment, the parameters of serum IFN did not change and remained within the normal range in almost all patients of groups 1 and 2. Spontaneous IFN reaction of lymphocytes in groups 1 and 2 was not altered.

During treatment, IFN-α synthesis by blood leukocytes in response to stimulation with NDV changed similarly in all groups. For instance, on day 8 this parameter tended to decrease, while by day 21 it increased more markedly in group 2. In healthy individuals, the titer of IFN-α was 80 U/ml. On day 8 of treatment, the production of IFN-γ by peripheral blood leukocytes in response to PHA stimulation only slightly varied between the groups and remained below the corresponding value in healthy individuals (32 U/ml). By the end of treatment, the level of IFN-γ production by group 2 patients tended to normal (25.7±1.9 U/ml). In group 1, IFN-γ producing capacity of leukocytes tended to decrease on days 8 and 21 of treatment (Fig. 1).

Study of the effect of therapy on parameters of IFN status showed different efficiency of recovery of IFN production depending on the initial values. In 66.7% patients of group 2, the recovery of IFN-γ production was most pronounced, if the initial production was slightly decreased (initial titer 16-32 U/ml, mean 18.7±1.2 U/ml). In patients with more pronounced initial disturbances in IFN-γ synthesis (titer 2-8 U/ml, mean 8.8±1.4 U/ml), the effect was absent. In the control group, IFN-γ-producing capacity of leukocytes against the background of therapy did not recover irrespective of the initial level of IFN-γ production (Fig. 2). Therefore, maximum efficiency of combined therapy with anaferon and acyclovir should be expected in patients with initially slightly lowered production of IFN-γ (titer 16-32 U/ml).

Evaluation of the expression of IFN-γ gene by mRNA production showed that in group 2 activity

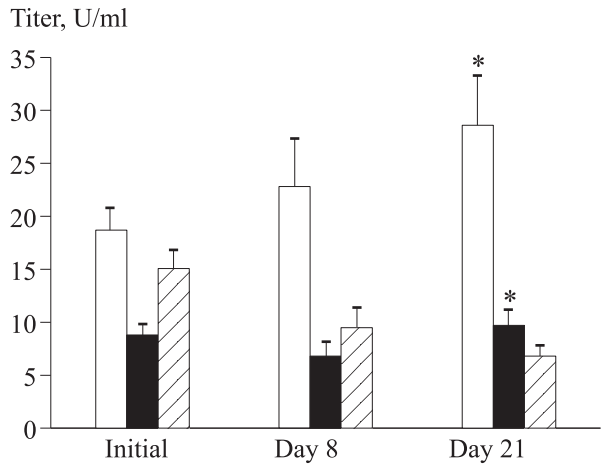

Fig. 2. Dynamics of parameters of IFN-γ production during treatment depending on their initial values in patients of groups 2 and 3. Initial titer of IFN-γ 16-32 U/ml (light bars) and 2-8 U/ml (dark bars); cross-hatched bars show data for group 3.

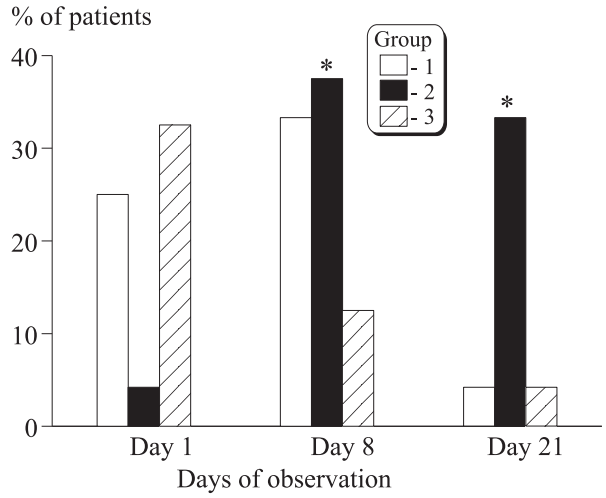

Fig. 3. Expression of IFN-γ mRNA during treatment.

of IFN-γ mRNA significantly increased by day 8 of treatment and remained at an appreciable level to the end of treatment. In other groups, this parameter decreased; in healthy volunteers IFN-γ mRNA was detected in 30% cases (Fig. 3).

Thus, the use of anaferon in complex therapy of patients with genital form of CHVI reduces the duration of general intoxication symptoms and local symptoms and shortens the period of crust formation. The positive clinical effect in patients was accompanied by changes in the following parameters: improvement of IFN-α- and IFN-γ-producing capacity and activation of IFN-γ mRNA. These data allow recommending anaferon as an immunomodulatory component of complex therapy and prophylactics of CHVI.

REFERENCES

1. F. I. Ershov, *Interferons anf Their Inductors (from Molecules to Drugs)* [in Russian], Moscow (2005).
2. F. I. Ershov, A. N. Narovlyanskii, and M. V. Mezentseva, *Tsitokiny i Vospalenie,* **3**, No. 1, 3-6 (2004).
3. L. A. Marchenko and A. V. Shurshalina, *Ginekologiya*, **2**, No. 3, 1-8 (2000).
4. T. B. Semenova, *Ros. Med. Zh*., **10**, No. 20, 924-930 (2002).
5. A. A. Khaldin and M. A. Samgin, *Ibid*., **12**, No. 4, 179-181 (2004).
6. L. N. Khakhalin, Herpes: *Unknown Epidemy (Pathogenesis, Diagnostics, Clinical Picture, and Therapy)* [in Russian], Smolensk, (1997), pp. 32-57.
7. A. E. Shul'zhenko and G. Kh. Vikulov, *Materia Medica*. No. 4, 60-79 (2003).
8. O. I. Epshtein, M. B. Shtark, A. M. Dygai, *et al., Pharmacology of Ultralow Doses of Antibodies to Endogenous Regulators of Functions* [in Russian], Moscow (2005).
9. M. H. Brentjens, K. A. Yeung-Yue, P. C. Lee, and S. K. Tyring, *Pharmacoeconomics*, **21**, No. 12, 853-863 (2003).
10. A. L.Cunningham and Z. Mikloska, *Herpes,* **8**, Suppl. 1, 6A-10A (2001).
11. S. Drake, S. Taylor, D. Brown, and D. Pillay, *Br. Med. J*., **321**, No. 7261, 619-623 (2000).
12. H. J. Field, *J. Clin. Virol.,* **21**, No. 3, 261-269 (2001).
13. R. L. Miller, M. A. Tomai, C. J. Harrison, and D. I. Bernstein, *Int. Immunopharmacol,* **2**, No. 4, 443-451 (2002).
14. S. Vollstedt, S. Arnold, C. Schwerdel, *et al., J. Virol*., **78**, No. 8, 3846-3850 (2004).