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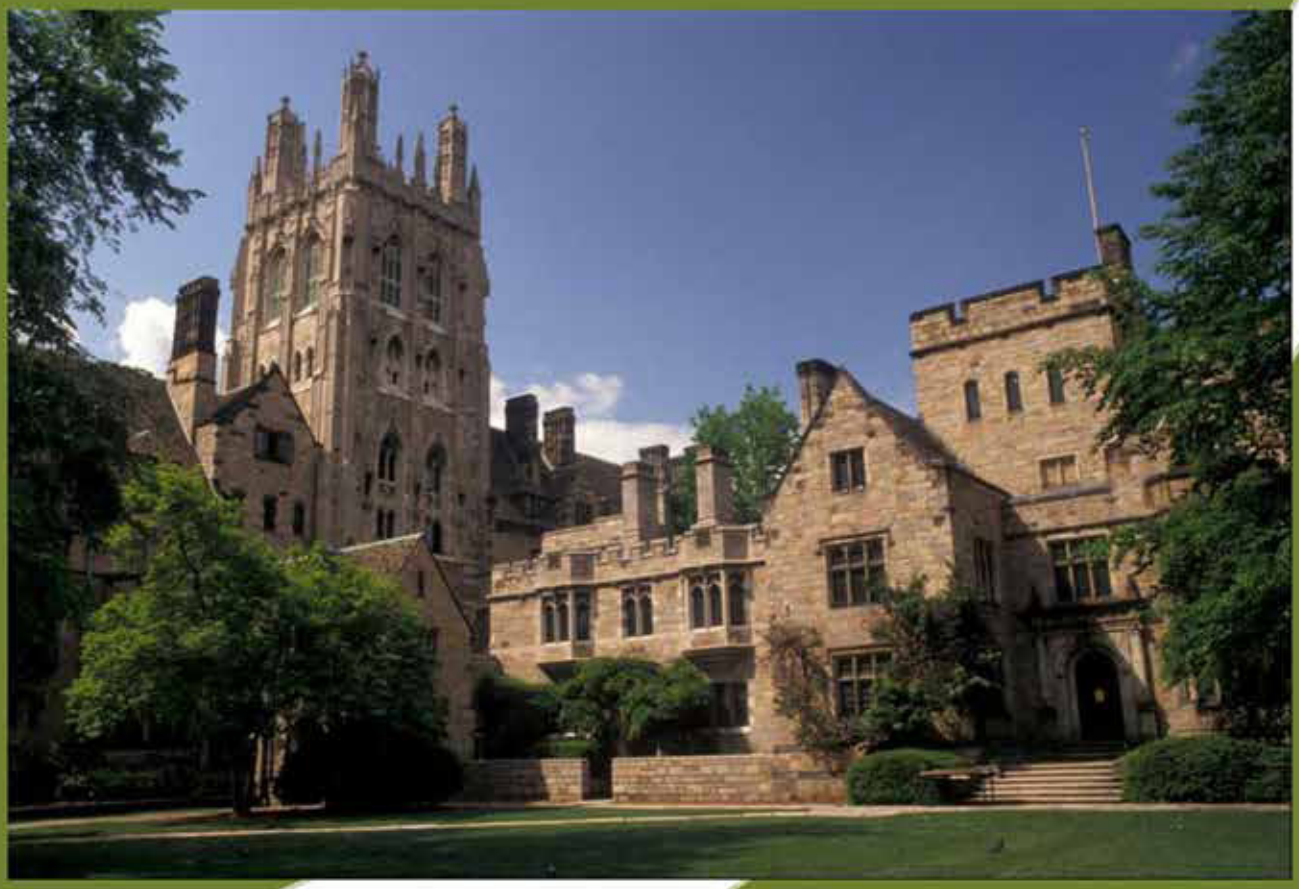

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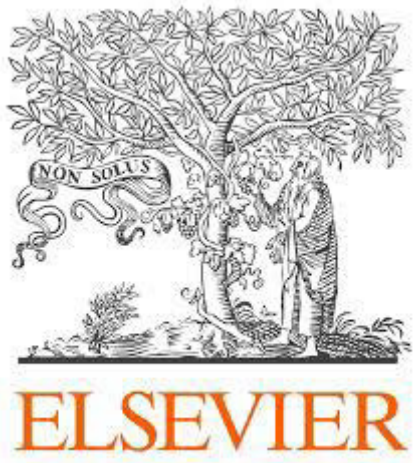

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Improvement of treatment of immunocompetent patients with herpetic infection

Abstract: On the basis of the clinical and immunological indices research of 84 immunocompetent patients with herpesvirus infections (labial recurrent herpes and/or stomatitis, the genital herpes and herpes zoster) it has been established that the standard therapy with daily reception of etiotropic medicine Valacyclovir 1,0 g 2 times a day within 7-10 days does not provide the powerful clinical effect.

Additional appointment of 6 subcutaneous injections of "Alokin-alfa" in 1 mg dose every other day allows to reduce statistically the duration of clinical manifestations of the genital herpes and herpes zoster, to diminish the frequency of recurrences and also to shorten the first recurrence duration after the specified treatment.

The combined treatment has essential impact on a number of immunological indices: the quantity of TCD3 + - and TCD4 + lymphocytes reaches almost normal rates, functional activity of mature T-lymphocytes and TCD16 + sells increases considerably.

Keywords: herpesvirus infections, immunological indices, treatment, "Alokin-alfa".

Introduction. According to WHO data, herpesvirus infections are among the most widespread and still difficultly controlled infections of a person. The end of the XX and the beginning of the XXI century are noted by significant increase of herpesvirus infections incidence around the world [2, 3].

Numerous researches have shown that more than 90% of a world's population at the age of 1-18 years old are infected with one or several strains of herpesvirus known nowadays (simple herpes of the 1st and 2nd types, a varitsella zoster, a cytomegalovirus, Epstein-Barre's (EBV) virus, herpes of the person of the 6^{th}, 7^{th} and 8^{th} types (HHV 6, 7, 8)) and have specific antibodies to them in blood [4-7].

For the treatment of patients with a herpetic infection it has been developed and used a large number of medicines, common fault of which is the absence of absolute clinical recovery effect. The effect of antiviral therapy is, as a rule, the short-term. The majority of antiherpetic medicines is the most effective during the acute period and does not influence remission duration greatly. Often the cancellation of preparations causes the recurrences with a former frequency. Another important problem is the emergence of resistant strains (including initially resistant) to basic antiviral therapy, particularly to acyclovir [8] that represents a serious problem at treatment of this pathology and can directly affect the course and outcome of the disease. This situation forces to develop new approaches of complex therapy of recurrent HVI actively.

The treatment of HIV-positive people with active HVI is declared by the Ministry of Health of Ukraine, protocol No. 182 from 13.04.2007 [9], according to which one of the etiotropic medicines is prescribed - an acyclovir, valacyclovir, famcyclovir. The main lack of a legal way of treatment is the temporary effect of a medicine.

Due to the relevance of a problem, an objective of this research is the studying of therapeutic efficiency of the new immunotropic medicine "Alokin-alfa" among patients with recurrent monoHVI infection.

Alokin-alfa (the registration certificate No. UA/8668/01/01 dated by 23.09. 2013) – is an anti-virus medicine of a new type. Active ingredient of the medicine is a cytokine-like peptide – Alloferon. The medicine has been patented in Ukraine, Russia, the USA, Japan, South Korea and the European Union, and is produced by "Biofarma" (Kiev) according to the license of the pharmaceutical company Geolik Farm Marketing Group - GFMG.

Alokin-alfa belongs to the immunotropic medicines. Its action is directed on strengthening of virus anti-genes and infected cells recognition by the natural killers, neutrophils or other effector systems of natural immunity, responsible for elimination of a virus. Such mechanism of action is actual while HVI treatment. As the researches of the last years have showed, various representatives of *Herpesviridae* protect a cell owner from destruction by cytotoxic lymphocytes, blocking activity of T-cells and natural killers. The fact of development of chronic infectious process at HVI is explained by it. Application of Alokin-alfa, perhaps, will improve recognitions of the diseased cells and will allow to suppress the centres of a virus replication.

Patients and methods. Total number of the examined and treated immunocompetent patients with recurrent herpetic infection was 84 persons.

For representative selection patients were divided into two groups. The first group included 40 immunocompetent patients with a herpetic infection. To these persons in addition to basic therapy within a week (daily reception of etiotropic medicine with the international unlicensed name (IUN) Valacyclovir - 1,0 g 2 times a day within 7-10 days) 3 subcutaneous injections of Alokin-alfa in a dose of 1 mg every other day were prescribed.

The second group included 44 patients with the specified basic therapy within a week. Immunological indices of 30 healthy persons were chosen as a control group. For this research patients were selected by the chance selection method. The period of supervision over patients consisted of treatment time and the subsequent outpatient supervision within 3 months.

Except complex clinical inspection, patients were provided with immunological research, definition of lymphocytes subpopulations, proliferative activity of T-lymphocytes, activity of NK-cells.

The research covered patients with a clinical picture of a herpetic infection in the acute period, no more than in 48 h from the moment of rash emergence. The test group included 23 patients with recurrent labial herpes and/or stomatitis, 21 persons with genital herpes and 40 persons with surrounding herpes. The average duration of disease was 4-8 years, average number of recurrences - 12 in a year.

The immediate treatment results were estimated due to the change of a herpetic infection recurrence duration. Recurrence duration (at achievement of a full reepithelialization) was estimated in days. The remote treatment results defined behind the course of a disease within 3 months of out-patient supervision: the number of the subsequent recurrences during the observed period, duration of remissions in days.

Statistical processing of the obtained results of research were carried out by means of the Windows, Word and Excel complex programs; STATISTICA 6.0 program with the use of a variation statistics method with reliability determination by means of a nonparametric Vilkokson's method for independent sets, or the dispersive analysis of Kraskela-Wallice. For the comparison of two independent groups U criterion of Mann-Whitney was used.

Discussion of research results. The use of Alokin-alfa in addition to basic therapy of patients with labial herpes and/or stomatitis, despite the lack of statistically significant changes, led to some duration reduction of HVI clinical manifestations during the treatment, and also to the first recurrence reduction after the treatment (P>0,05).

However, while the assessment of clinical manifestations duration of the genital and herpes zoster during the treatment, it was found its statistically reliable reduction during the combining basic therapy with Alokin-alfa in comparison with treatment only by Valacyclovir - respectively (4,3 ± 0,5) against (6,6 ± 0,8) days and (5,9 ± 0,6) against (8,6 ± 1,2) days (in both cases P<0,05).

The next observations of patients with genital and the herpes zoster also convincingly show the advantage of combining basic therapy with Alokin-alfa. So, the frequency of recurrence decreased, the duration of the first recurrence after the specified treatment was shortened: (3,9 ± 0,6) against (5,7 ± 0,6) days at genital herpes and (4,6 ± 0,7) against (7,8 ± 1,3) days at the herpes zoster (in both cases P<0,05). Basic therapy of control group patients did not influence the duration of recurrence significantly.

Under the influence of the approved methods of therapy recurrence duration during the treatment decreased, and also duration of clinical manifestations at the emergence of the first recurrence after therapy was shortened. The use of Alokin-alpha in addition to basic therapy provided the shortening of recurrence during the treatment and the first recurrence after therapy was more considerable in comparison with the treatment by Valacyclovir. In particular, basic therapy at labial herpes shortened the specified duration of recurrence by 27,3%, combined - by 56,7%, at genital herpes - by 38,8% and 55,7%, at the herpes zoster - by 31,6% and 61,7% respectively.

Three-months out-patient supervision over patients who received only basic therapy indicated only a tendency to reduction of the subsequent recurrence frequency (P>0,05).

At the same time the use of Alokin-alpha in addition to basic therapy provided the increase of remission duration and reliable frequency reduction of the subsequent recurrence of all clinical forms of a herpetic infection (in all cases P<0,05). The approved treatment had essential advantages over the basic therapy at patients with labial herpes and/or stomatitis, and patients with genital herpes as it

provided statistically significant reduction of the recurrence number within 3 months after the treatment: 0,63 ± 0,20 against 1,92 ± 0,41 times and 0,60 ± 0,28 against 1,91 ± 0,33 times respectively (in all cases $P<0,05$).

Thus, the use of "Alokin-alfa" medicine in complex therapy of patients with recurrent labial herpes and/or stomatitis, the genital and herpes zoster by criteria of the clinical efficiency provided considerable therapeutic effect in comparison with Valacyclovir that was applied in control group.

After the treatment of patients with herpesvirus infections by Valacyclovir, none of the considered immunological indices had a reliable difference in comparison with patients before treatment. At the same time, the quantity of TCD3 + lymphocytes grew to almost normal rates at genital herpes - (1513,8 ± 304,3) cells / mcl against (1403,4 ± 199,2) cells / mcl respectively (at healthy persons - (1993 6 ± 188,9) cells / mcl). But only at the herpes zoster these indices continued to remain authentically lower - (1371,7 ± 239,2) cells / mcl than in norm ($P<0,05$).

Authentically lowered in the herpesvirus infections acute period, the number of T-helpers at the genital herpes and herpes zoster under the influence of treatment by Valacyclovir significantly increased, practically reaching values of healthy people - (1245,6 ± 94,1) and (1157,8 ± 146,3) cells / mcl at norm (1422,5 ± 95,7) cells / mcl ($P>0,05$).

Combining of the antiviral therapy with Alokin-alpha provided substantial increase of mature T-lymphocytes functional activity by 92,6% at genital herpes - (2703,4 ± 326,2) against (1403,4 ± 199,2) cells / mcl before treatment and by 112,1% at the herpes zoster - (2618,1 ± 250,4) against (1234,6 ± 104,5) cells / mcl before treatment (in all cases $P<0,05$).

The combined treatment provided normalization of the TCD4 level + lymphocytes which at the herpes zoster statistically exceeded indices at patients before treatment - (1239,9 ± 130,6) against (1157,8 ± 146,3) cells / mcl respectively ($P<0,05$).

Signs of Alokin-alfa negative influence on the immune status indices were not established though it should be noted that the T-suppressors level under the influence of the combined therapy was characterized by a tendency to growth, and at the herpes zoster it authentically exceeded the corresponding indices at patients before treatment - (616,9 ± 74,5) against (423,8 ± 41,1) cells / mcl respectively ($P<0,05$).

It is possible to assume that the approved immunotropic medicine possesses a certain stimulation of cytotoxic T-effectors (TCD8 + cells).

Alokin-alpha influence was also characterized by a considerable strengthening of TCD16^{+} cells activity, which are natural killer markers and are responsible for an antibody-independent specificity – cumulative specific and nonspecific cytotoxicity. So, at patients with genital herpes this index was (492,8 ± 43,9) cells / mcl that by 70,7% exceeds indices in the acute period and by 48,6% after traditional treatment by Valacyclovir. At the herpes zoster such excess was 73,6 and 52,6% respectively (in all cases $P<0,05$). Thus the TCD16 level + cells always remained within normal indices.

Persistent herpesviruses adapted to reducing interaction with the immune system of the owner that allows them to replicate and circulate among healthy people.

Persistency is reached by the combined activity of the program of an expression of virus latency gene and by the immunosuppressive molecules production. It allows a virus to avoid recognition by NK cells and cytotoxic T-lymphocytes [12].

The obtained data show that Alokin-alfa is a selective specific stimulator of natural killers' functional activity that is connected with the direct strengthening of these cells cytotoxic activity. Such mechanism of action is especially actual treating patients with HVI.

Thus, the obtained data confirm antiviral and immunomodulating effects of Alokin-alfa, that in combination with traditional antiherpetic therapy provides powerful clinical effect and significant improvement of cellular immunity indices of patients with herpesvirus infections. This fact favourably distinguishes such treatment from traditional therapy by Valacyclovir only.

Conclusions:

1. Only basic therapy of immunocompetent patients with herpesvirus infections (recurrent labial herpes and/or stomatitis, the genital and herpes zoster) by daily reception of etiotropic medicine Valacyclovir in a dose of 1,0 g 2 times a day within 7-10 days does not provide powerful clinical and immunological effect.

2. The combination of the specified treatment with 6 subcutaneous injections of "Alokin-alfa" in a dose of 1 mg every other day allows to reduce statistically clinical

manifestations duration of the genital herpes and herpes zoster, to reduce the recurrence frequency of various clinical forms of a herpetic infection, and also to shorten the duration of the first recurrence after the specified treatment (in all cases P<0,05).

3. The tested combined treatment has the essential impact on a number of immunological indices. So, at genital herpes the TCD3 + and TCD4 + lymphocytes quantity grows to almost normal indices, the functional activity of mature T-lymphocytes considerably increases (by 92,6% at genital herpes and by 112,1% at the herpes zoster - in all cases P<0,05). Such therapy also provides considerable strengthening of TCD16 + cells activity: at patients with genital herpes this indicator exceeds rates in the acute period of the illness by 70,7% and by 48,6% after the traditional treatment by Valacyclovir. At herpes zoster such excess formed 73,6% and 52,6% respectively (in all cases P<0,05). Thus the TCD16 + cells level always remained within normal indices.

4. Parenteral Alokin-alpha application did not give allergic or other collateral reactions, it was well tolerated by patients.

5. The practicing doctors do not need to carry out the immunological researches for making decision about Alokin-alfa prescribing as on the basis of the herpesvirus infections pathogenesis studying it was stated the oppression of cellular reactions and the decrease of the cytotoxic lymphocytes activity. It should be noted that factors of nonspecific resistance (interferon and cytotoxic lym-phocytes) are the main targets of Alokin-alfa in the patient's organism.

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