Effects of Polyoxidonium on Phagocytic Cell Functions. Experimental and Clinical Estimation of Potential Inclusion of Polyoxidonium in Complex Therapy in Penetrating Eye Injury

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The purpose of present paper is the investigation of polyoxidonium effects on the functions of circulating pool of phagocytic cells and the estimation of its potential inclusion in complex therapy in penetrating eye injuries. On experimental model of eye injuring in rats there has been established that polyoxidonium attenuated some negative glucocorticoid effects on phagocytic cells. Inclusion of polyoxidonium in complex therapy optimized the course of injuring process according to parameters of the least infiltration of damage area with immunocompetent and effector cells, scar structure and other parameters. The stimulation of neutrophil, and in a less degree of eosinophil phagocytosis, was demonstrated at concentration range from 10^{-11} to 10 μg/ml under conditions of 1 h drug preincubation *in vitro* with blood cells of healthy people. At concentrations 10^{-11}-10^{-8} μg/ml polyoxidonium caused a slightly stimulated effect on the parameters of monocyte phagocytosis. As a whole the results obtained show that further studies are promising for potential use of polyoxidonium in complex therapy for penetrating eye injuries.

INTRODUCTION

Last years are characterized by the appearance in clinical practice of new home produced immunocorrection drugs with selective action on separate compartments of immune system. One of the brightest example of such an immunomodulator is polyoxidonium which was synthesized from aliphatic polyamines at the Institute of Immunology, Ministry of Health of Russian Federation. The development of this drug was one of the logical outcomes of complex investigations dealing with search of artificial antigens on the base of synthetic polyelectrolytes and with phenotypic correction of immune response which were begun in the middle of the 70ties under the supervision of academician of RAS R.V. Petrov and academician of RAMS R.M. Khaitov [1-5]. It has been shown that *in vivo* polyoxidonium stimulates ingestive and bactericidal activity of macrophages, activates immune response and cooperation between T and B lymphocytes in mice, normalizes the number of $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD22^+$ lymphocytes, enhances leukocyte phagocytic capacity, and at the concentration 100 μg *in vitro* stimulates human neutrophil activities: adhesion, values of NBT-test, luminol-dependent chemoluminescence, production of cationic proteins, but inhibits their chemotaxis [6].

Present work reviews a part of investigations conducted by us in the field of possible use of

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polyoxidonium for correction of disturbances of immune system function in traumatic injuries of eyes.

Penetrating eye wound is the most main cause resulting in decrease and loss of vision [7, 8]. In such an injury the combination of deficiency of T cell compartment with autoimmune disorders takes place [9-11]. The feature of given pathology, unlikely from trauma of other organs, is a potential development of autoimmune damage of healthy eye (sympathetic ophthalmia), and therefore immunodeppressants (for example, glucocorticoids) are administered together with antibiotics and anti-inflammatory drugs [7, 12]. Since the investigation of various compartments of immune system is carried out on the background of treatment, it is not always easy to discriminate the changes caused due to trauma from those which appeared as a result of treatment. The latter may be possible only in experimental conditions and it is needed for the development of pathogenically valid methods of correction of immune disorders in penetrating wounds of eye.

Phagocytic cells, granulocytes and mononuclear cells, play an important role in the course of traumatic process and in the development of inflammation with subsequent regeneration. The functional changes of these cells in peripheral blood in trauma may be related both to factors of systemic regulation, and to dynamics of local reactions [13, 14]. In trauma of various localization the inhibition of clearance function of mononuclear phagocyte system is noted, pertinent first of all to the depression of phagocytic activity of Kuppfer cells [13-17]. The changes of function of other phagocytic cells, for example granulocytes, are not sufficiently studied. Any trauma is characterized by development of neutrophil leukocytosis [14]. However, the association of the degree of neutrophil leukocytosis with the changes of neutrophil function is not always fairly clear. Particularly, in the case of acute stress there is a dissociation between relative and absolute parameters of bactericidal and ingestive functions of peripheral blood neutrophils [18, 19].

The purpose of present paper is the investigation of polyoxidonium effects on the functions of circulating pool of phagocytic cells and the estimation of its potential inclusion in complex therapy in penetrating eye injuries. One of the main tasks was the estimation of dynamics of oxidative potential of circulating pool of phagocytic cells in experimental penetrating eye injury under various therapy protocols, including polyoxidonium. In addition, the relationship dose-effect of polyoxidonium action on phagocytic activity of peripheral blood neutrophils, eosinophils and monocytes of healthy donors was studied that is required for the development loading tests with polyoxidonium for prediction of its effect *in vitro*. The work gives the first results of clinical use of polyoxidonium in the complex therapy of penetrating eye injuries.

MATERIALS AND METHODS

Estimation of Polyoxidonium Effects in an Experimental Penetrating Eye Injury

The experiments were carried out on 41 male rats of Wistar strain with weight 200.5±6.64 g (M±m). All animals were divided into 4 groups. Rats of the 1st group (11 animals) were subjected to a penetrating injury of the right eye. Animals of the 2nd group (10 animals) were subjected to the similar procedure with the subsequent standard therapy, extrapolated from the protocols used in clinic (ampicillin 12,500 U/kg of a body weight subcutaneously 2 times per day, gentamycin 1.5 mg/kg of a body weight subcutaneously 2 times per day, dexamethasone 0.1 mg/kg of a body weight subcutaneously 1 time per day in the morning, sodium diclofenac 0.5 mg/kg of a body weight subcutaneously 2 times per day). Animals of the 3rd group (10 animals) were exposed to a penetrating wound of an eye with the subsequent standard therapy in combination with polyoxidonium immunocorrection (0.1 mg/kg subcutaneously in the morning before injuring, and also on the 2nd, 4th, 6th and 8th day after injury). Rats of the 4-th group (10 animals) were subjected to the similar procedure with the subsequent monotherapy with polyoxidonium. Medicinal preparations in the 2nd, 3rd and 4th groups were administered in 7 hours after injuring, therefore dynamics of parameters before this time (pooled initial background, 180 and 360 min after a trauma) is analyzed in the combined group (differences between groups used for pooling, were not revealed in the specified time intervals according to

Student's *t*-criterion). Samples of blood in volume 0.55 ml were taken from tail vessels in Eppendorf's tubes with heparin (50 U/ml of blood) before drawing a trauma (parameters of an initial background), and also in 180 min, 360 min, 1, 2, 3, 5, 7 and 12 days after injuring (the control of erythrocyte number in peripheral blood has shown absence of statistically significant changes of this parameter in the 1st group). Penetrating injuring of an eye rendered in the specially designed device-holder with a microblade in the area of limb (boundary area between sclera and cornea). At the same time there was an injuring of cornea, sclera and vascular load. The leak of intraocular liquid and vitreous humor was observed in all cases after a trauma. The damage of lens, extravasation of blood to the front chamber and vitreous humor took place in some cases. The local purulent inflammation with presence hypopyon has developed in the majority of animals in time from 2 about 7 days. Each sample of blood was studied by a set of microassays for estimation of functions phagocytic cells. In the given paper the changes concerning only parameters of bactericidal function of phagocytic cells will be considered in spectrophotometric variant of the test with nitroblue tetrazolium (**NBT-test**). The modified method was used [20]. Briefly, 25 μl of whole blood, 12.5 μl of opsonized zymosan (Sigma, USA, concentration - 3.0 mg/ml), 12.5 μl of nutrients (culture medium 199 with addition of 2 mm L-glutamine and 10 mm HEPES-buffer) and 25 μl of NBT 0.15% solution in 0.15 M sodium chloride solution were mixed in stimulated sample. In spontaneous test the same components were mixed, but instead of zymosan 12.5 μl of 0.15 M sodium chloride solution was added. The same ingredients were put to control samples, except for a NBT solution, instead of which 0.15 M sodium chloride solution was added. After incubation for 30 min at 37°C the reaction was stopped by addition of 0.5 N hydrochloric acid in test tubes. The further course of procedure did not differ from described one [20], except that volume of all reactants was increased in 1.5 times. The results were expressed as absolute numbers of optical density (using 25 μl of blood), and also as symbolically relative units. The latter's were calculated according to the equation: (A/B) x 10^6, where A - extinction of test-sample against the control registered by spectrophotometer at 710 nm; B - absolute content of phagocytes in 1 μl of blood (neutrophils, monocytes and eosinophils).

Estimation of Polyoxidonium Influence on Phagocytic Leukocyte Activity of the Healthy People in vitro

Influence of polyoxidonium on phagocytic activity of neutrophils, eosinophils and monocytes of peripheral blood was studied in 13 practically healthy men in the age of from 16 till 44 years old. The modified method was used [21]. In microtubes with an anti-adhesive coating 25 μl of a heparinized blood (heparin was added at the dose of 50 U/ml) was mixed with 12.5 μl of polyoxidonium, diluted in medium 199 with addition of 10 mm HEPES-buffer and 2 mm of a L-glutamine (final concentrations of a preparation - from 100 μg/ml to 10^{-11} μg/ml with a 10-fold increment). Test-samples were incubated within 1 hour at 37°C. Then the objects of phagocytosis were embedded (repeatedly washed fomalinized sheep red blood cells, prepared with medium of the same composition in concentration $200x10^6$/ml). Test-samples were incubated for 20 min at 37°C. Earlier mentioned complex of parameters was used for differential assessment of phagocytic activity of neutrophils, monocytes and eosinophils on smears, stained by Romanowsky-Guimza [22]. Controls were test-samples with preincubation, where the same volume of nutrient medium, instead of a medicinal preparation, was put in. The additional controls were test-samples without preincubation, depicting a level of initial phagocytosis.

Statistical Analysis

Unpaired and paired Student's *t*-criteria, as well as non-parametrical Wilcoxon's W-criterion of the sum of ranks and paired signed Wilcoxon's T-test were used for assessment of difference significance [23].

RESULTS

Influence of Polyoxidonium on NBT-Test Parameters in an Experimental Penetrating Eye Injury

Dynamics of absolute parameters of the NBT-test is depicted on **Figure 1** and **2**. In 180 min

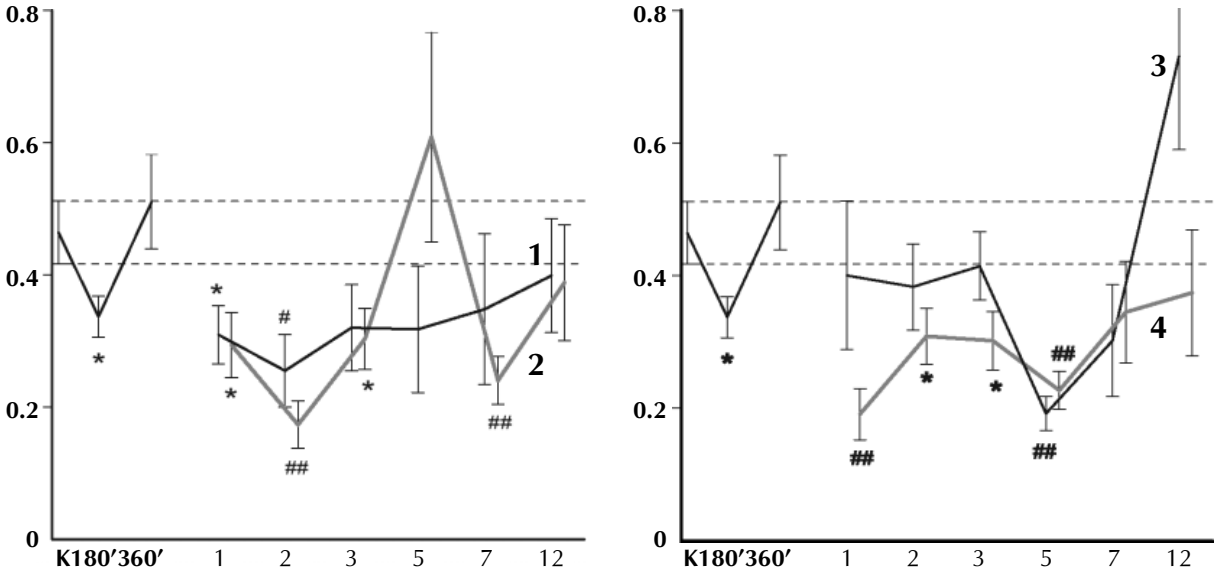
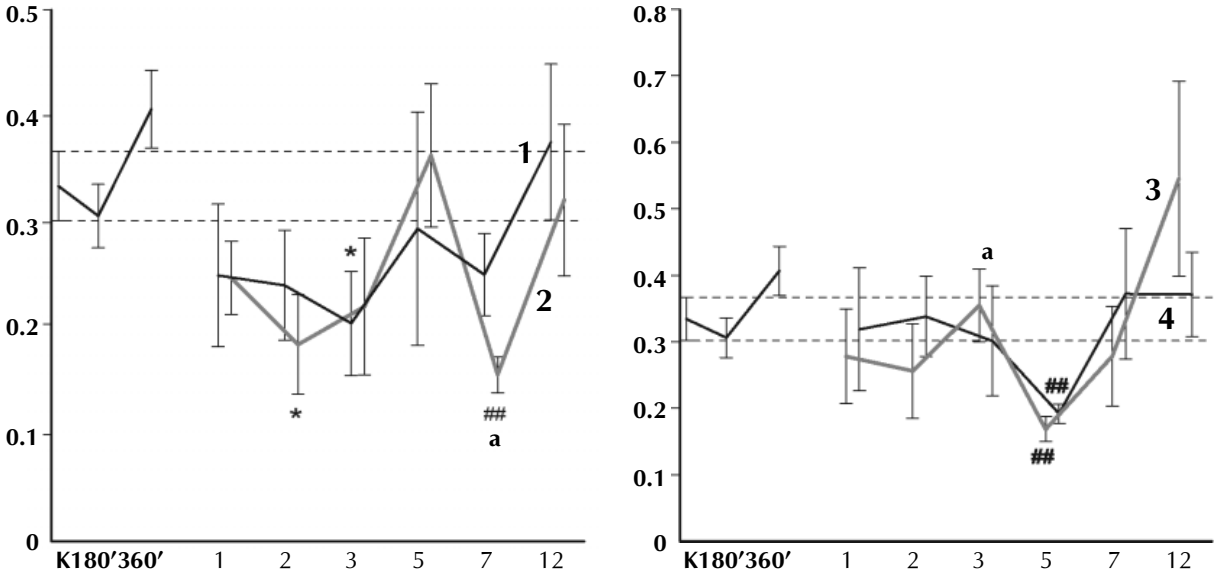


Figure 1. Dynamics of absolute parameters of NBT-test (stimulated variant)

1 – the 1st group; **2** – the 2nd group; **3** – the 3rd group; **4** – the 4th group. **Axis X:** K180′ and 360′ - the parameters of combined group: initial background (control), 180 and 360 min after injuring respectively; 1, 2, 3, 5, 7, 12 days – time intervals after injuring in the 1st and 2nd groups. **Axis Y:** absolute values of extinction per 25 ml of blood (M±m). * - $p<0.05$ compared to control; # - $p<0.01$ compared to control; ## - $p<0.001$ compared to control; a – $p<0.05$ compared to the 1st group; b – $p<0.01$ compared to the 1st group; c – $p<0.001$ compared to the 1st group (using unpaired Student's *t*-criterion).

after injuring the decrease of bactericidal potential of phagocytic cells in stimulated variant of test and its restoration up to an initial level 360 min later were observed for the pooled group. In test-samples without addition zymosan (spontaneous variant) the absolute parameters did not statistically significant change in these time periods ($p > 0.05$). In 24 hours after drawing a trauma the animals that not received therapy (1st group) have revealed the decrease of absolute parameters of NBT-test in

Figure 2. Dynamics of absolute parameters of NBT-test (spontaneous variant). Designations are the same that in Fig. 1.



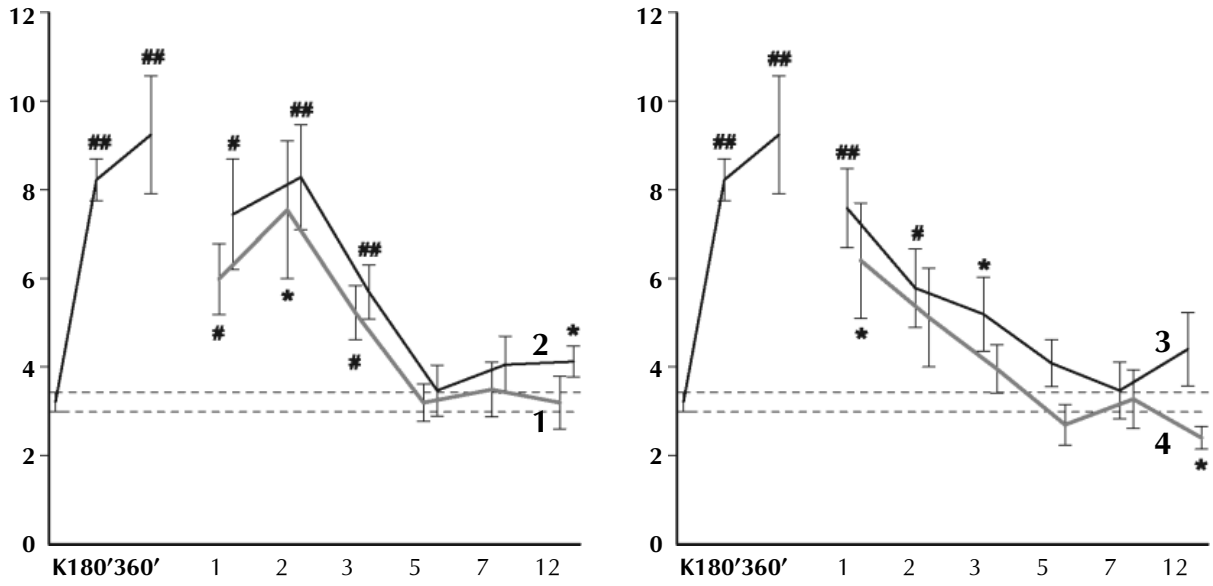


Figure 3. Dynamics of the total number of neutrophils, monocytes, eosinophil and basophils
Axis Y: absolute cell numbers (x 10^3) in 1 ml of blood (M±m). The rest designations are the same that in Fig. 1.

stimulated variant which did not statistically significant differ from the control, beginning from 3 day after injuring. In spontaneous variant of NBT-test the production of the active forms of oxygen in absolute expression was reduced on the 3rd day. In penetrating eye injury the decrease of absolute parameters of the NBT-test develops on a background of substantial growth in blood of the total content of cells participating in restoration NBT, that is all the granulocytes and monocytes (**Figure 3**). It is possible, that dissociation between the absolute parameters of the NBT-test and the number of cells participating in restoration NBT is connected to immigration of relatively immature phagocytes from bone marrow to blood circulation (confirmation to that is the appearance in all the animals of neutrophil leukocytosis with rod nuclear shift to the left). It is impossible though to exclude the opportunity of migration of the most active phagocytes from the blood circulation to the focus of traumatic inflammation, and also direct inhibiting effect of hormones, formed under of traumatic stress conditions, on the function of phagocytic cells. Revealed in animals of the 1st group statistically significant (by unpaired Student's *t*-criterion) increase of concentration glucose in 360 min, 1, 2, 7 and 12 days after drawing a trauma, apparently, connected with stress release of contra-insulin hormones, and also statistically significant increase of adrenal weight (12 day) is the confirmation of the latter assumption. However it should be emphasized that the activation of absolute parameters of the NBT-test on 3, 5 day which is characteristic for immobilization stress described earlier [18, 19], is absent, that is under conditions of a trauma the depression of functions phagocytic cells dominates.

The animals with standard therapy (2nd group), have more expressed decrease of absolute parameters of the NBT-test during the first days of a trauma (statistically significant more expressed in relation to the control, than in 1st group, and proceeding about 3 day in stimulated variant of the test) and, besides it, development of the second wave of decrease of these parameters on the 7th day (Fig. 1 and 2). Thus, the decrease of bactericidal potential of phagocytic cells in absolute numbers is more pronounced on a background of standard therapy. It, apparently, is connected to inclusion in it synthetic glucocorticoid dexamethasone, that under conditions of clinic is dictated by necessity of prevention autoimmune eye damages [7, 12].

In animals that received standard therapy in a combination with polyoxidonium (3rd group), the reduction of absolute parameters of the NBT-test in stimulated and spontaneous variants of the test was noted only on the 5th day (Fig. 1 and 2). The administration of immunomodulator not only prevents the decrease, connected to standard therapy, of absolute parameters of the NBT-test on the 7th day (as it is observed in the 2nd group), but also cancels depression of oxygen-dependent mechanisms of bactericidal activity for the first days of a trauma (that is characteristic for 1st group). As a whole the development of less expressed depression of absolute parameters of the NBT-test is characteristic for 3rd group of animals, than for 1st and 2nd groups. It may be pertinent to inclusion of polyoxidonium into therapy protocol. That is, the drug cancels development of depression of the NBT-test in trauma under conditions of the combined therapy.

However, more long (statistically significant in the 1st, 2nd, 3rd and 5th days) decrease of absolute parameters in stimulated variant of the NBT-test was noted after administration of only single drug polyoxidonium (the 4th group) (Fig. 1). In other words, the drug enhanced traumatic depression of oxygen-dependent restoration NBT, triggered by opsonized zymosan through receptors for complement components (as it is known, presented on phagocytic cells by integrins CD11b/CD18 (CR3), CD11c/CD18 (CR4), and also CD35 (CR1), performing functions as receptors for C3b, C3bi, C4b components). On the contrary, in spontaneous variant of the test (Fig. 2) the depression of absolute parameters as well as in the 3rd group was marked only on the 5th day.

Summing up the characteristic of changes of absolute parameters of NBT-test, it is important to note, that despite of increase of total granulocyte and monocyte numbers in blood after eye injuring, the absolute parameters of bactericidal potential of these cells were reduced. The standard therapy (apparently, due to the action of glucocorticoids) heightened depression of bactericidal potential of phagocytic cells. The simultaneous administration of polyoxidonium in the 3rd group reduced unfavorable action of glucocorticoids. However polyoxidonium itself (in the 4th group) increased and extended the development of depression of bactericidal potential of phagocytic cells, caused by the trauma, in stimulated variant of the NBT-test.

More distinctly, the deficiency of oxygen-dependent mechanisms of bactericidal activity of circulating pool of phagocytic cells in trauma is appeared to be manifested in analysis of relative parameters of the NBT-test (**Figure 4** and **5**). Decline of relative parameters of the NBT-test (that is extinction parameters, calculated per 25 x 10^6 of sum of neutrophils, eosinophils and monocytes) both in stimulated, and in spontaneous variant was observed in 180 minutes after drawing a trauma. In animals without therapy (1st group), the relative parameters of stimulated and spontaneous variants of the NBT-test remained reduced up to 3rd day. Unlike to the 1st group, animals with standard therapy (the 2nd group) have revealed more marked development of the 1st wave of reduction of relative parameters of the NBT-test (on the 1st-3rd days), and also development of the 2nd wave of decrease of relative parameters both in stimulated, and in spontaneous variants on the 7th day. Addition of polyoxidonium to standard therapy (3rd group) changed in some degree the dynamics of decrease of relative parameters of the NBT-test both in spontaneous, and in stimulated variants. Therein, the decrease of parameters on the 7th day of a trauma, which is characteristic for the 2nd group, was not found, but the inhibition was revealed on the 5th day. On a background of polyoxidonium monotherapy (the 4th group) alignment of traumatic depression of relative parameters of the NBT-test in spontaneous variant was noted (statistically significant decrease has been revealed only on the 3rd day of a trauma).

However the inhibition of relative parameters of stimulated variant of the NBT-test was longer, than in the 1st group.

Thus, the standard therapy increased depression of relative parameters of the NBT-test. This effect, apparently, first of all is mediated by dexamethasone. Treatment of animals with polyoxidonium in combination with standard therapy slightly changed dynamics of depression of relative parameters of the NBT-test and canceled the inhibition of bacte-

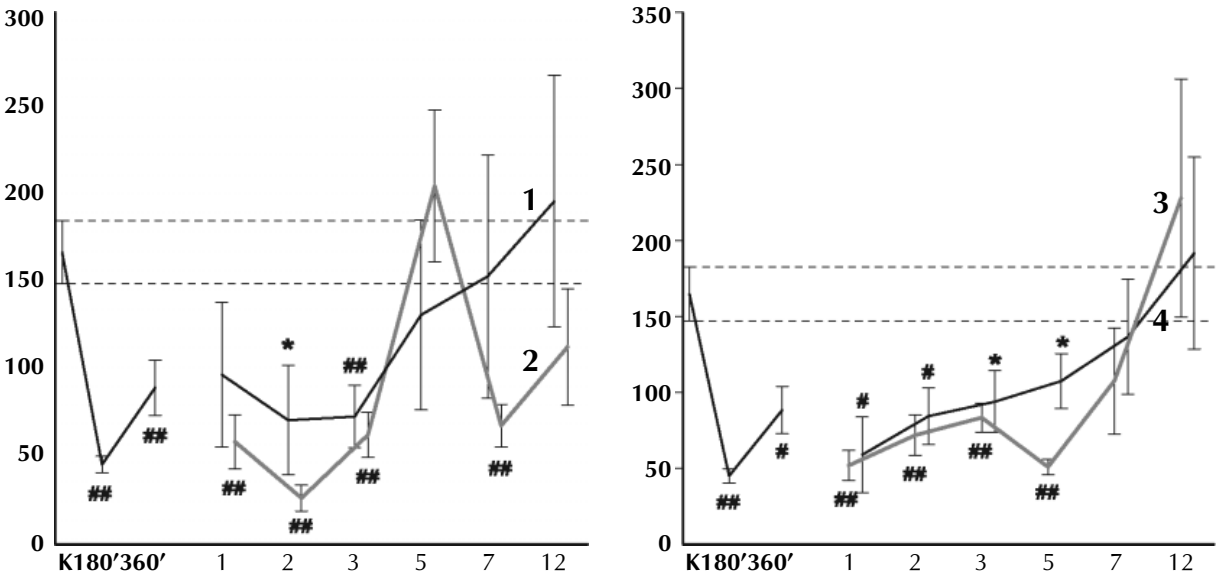
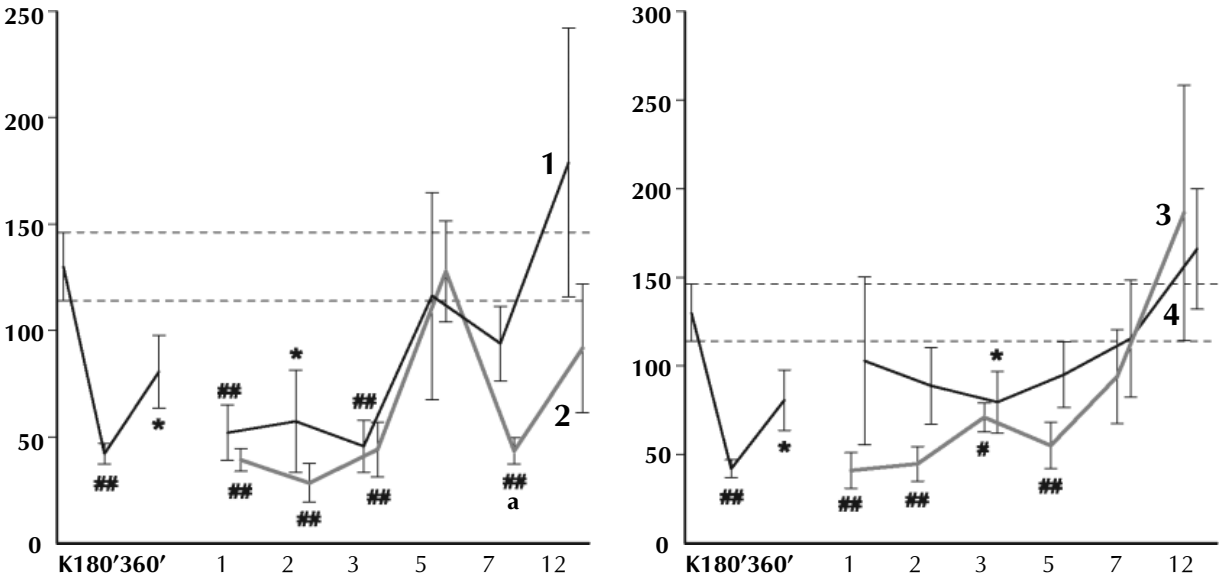


Figure 4. Dynamics of relative parameters of NBT-test (stimulated variant)
Axis Y: relative extinction parameters, expressed in arbitrary units (M±m). The rest designations are the same that in Fig. 1.

ricidal potential of phagocytes, caused by standard therapy on the 7th day of a trauma. The administration of only monotherapy with polyoxidonium considerably reduced the duration of decrease of parameters of the NBT-test in spontaneous variant, but increased it in stimulated one.

Other negative effect of standard therapy, administered for the 2nd group, was the development of leukopenia and absolute lymphopenia at the 1st, 5th, 7th and 12th days of a trauma ($p < 0.05$ to the control). Besides it, the decrease of the total number of nucleated cells in thymus and spleen was found out

Figure 5. Dynamics of relative parameters of NBT-test (spontaneous variant)
Axis Y: relative extinction parameters, expressed in arbitrary units (M±m). The rest designations are the same that in Fig. 1.



on the 12th day of a trauma ($p < 0.05$ to the control), also, apparently, connected with effect of dexamethasone. Administration of polyoxidonium in combination with standard therapy partially canceled development of leukopenia and decrease of cellularity of thymus, but not spleen. Administration of only one drug, polyoxidonium, in the 4th group resulted in substantial growth of thymus cellularity on the 12th day ($p < 0.05$ to the control), however the drug provided statistically significant decrease of the total leukocyte number (due to lymphocyte reduction) on the 5th and 7th days.

Thus, after simultaneous use of polyoxidonium with glucocorticoids and other preparations of basic therapy negative effects of the latter are partially compensated, though the drug itself extends traumatic depression of absolute and relative parameters of stimulated (but not spontaneous) variant of the NBT-test. Since in penetrating eye injuries the probability of development of autoimmune damages is high, glucocorticoids have been and have remained the basic component of therapy. The addition of polyoxidonium to this therapy allows even partially to lower side effects of glucocorticoids. As the changes of functional parameters of phagocytic cells in peripheral blood only reflect real processes played in the area of damage during the development of local inflammatory response, pathomorphological investigations of injured and intact eye give important data for estimation of efficiency of therapy. Such results have shown extremely unfavorable course of wound process in an injured eye in animals without any treatment.

The moderate amount of homogeneous deposits of light pink color was determined in chambers of an uninjured eye on the 12th day of experiment in rats of the 1st group. Pronounced inflammatory infiltration with lymphocytes, plasma cells, polymorphonuclears, single eosinophils was noted for all cases in various structures of an injured eyeball. In all studied eyes the wound is covered with multilayer flat epithelium of various thickness, the scar is formed with single fine vessels, fibroblasts, histiocytes, lymphocytes, plasma cells. Proliferation of keratoblasts, the abundance of lymphocytes and stroma edema are observed around of an area of damage. In the field of a wound there are commissures with iris with marked plasmaleukocytic infiltration with single eosinophils, abundance of full-blooded thin-walled vessels. In two cases the pronounced lymphocyte-polynuclear cell infiltration is revealed in the field of damage, of iris with its destruction, of ciliary body, of capsule of crystalline lens. The destruction and the formation of commissures took place in 6 eyeballs, alongside with marked protein exudation in anterior and posterior chambers, and in vitreous humor. On the 12th day only traces of homogeneous deposits of light pink color were determined in an uninjured eye in rats of the 2nd group. In an injured eye the wound is covered with multilayer flat epithelium of various thickness, the scar is formed with few fine full-blooded vessels, fibroblasts, lymphocytes. There is mild lymphocytic-plasmacytic infiltration in iris and ciliary body.

Iris commissures are present with its damage in which may be seen fine collagen fibers, fibroblasts, small numbers of lymphocytes, histiocytes, plasma cells. The anterior chamber is of non-uniform depth with protein exudation, which also may be determined in the posterior segment of an eye. The changes were not found in uninjured eyeballs in rats of the 3rd and the 4th groups on the 12th day. In injured eyes of rats of the 3rd group in all cases the wound was epithelized in association with keratoblast increase and normal, but loose arrangement of collagen fibers. Weak (minimal) infiltration may be seen in ciliary body, iris, episclera. In the injured eyes of rats of the 4th group the area of damage is covered multilayer flat epithelium of various thickness. Mild lymphocytic-plasmacytic infiltration was determined with single leukocytes in structures of eyeball, especially of ciliary body, iris, episclera.

In vitro Influence of Polyoxidonium on Phagocytic Activity of Peripheral Blood Neutrophils, Eosinophils and Monocytes in Healthy People

Since polyoxidonium is expected to be promising drug for its inclusion in complex therapy in penetrating eye injuries, it is necessary to develop *in vitro* tests for prognosis of its effects *in vivo* with the subsequent estimation of usefulness of such tests in patients. Load tests with polyoxidonium may be called as examples of such tests, in particular, the study of its influence on phagocytic

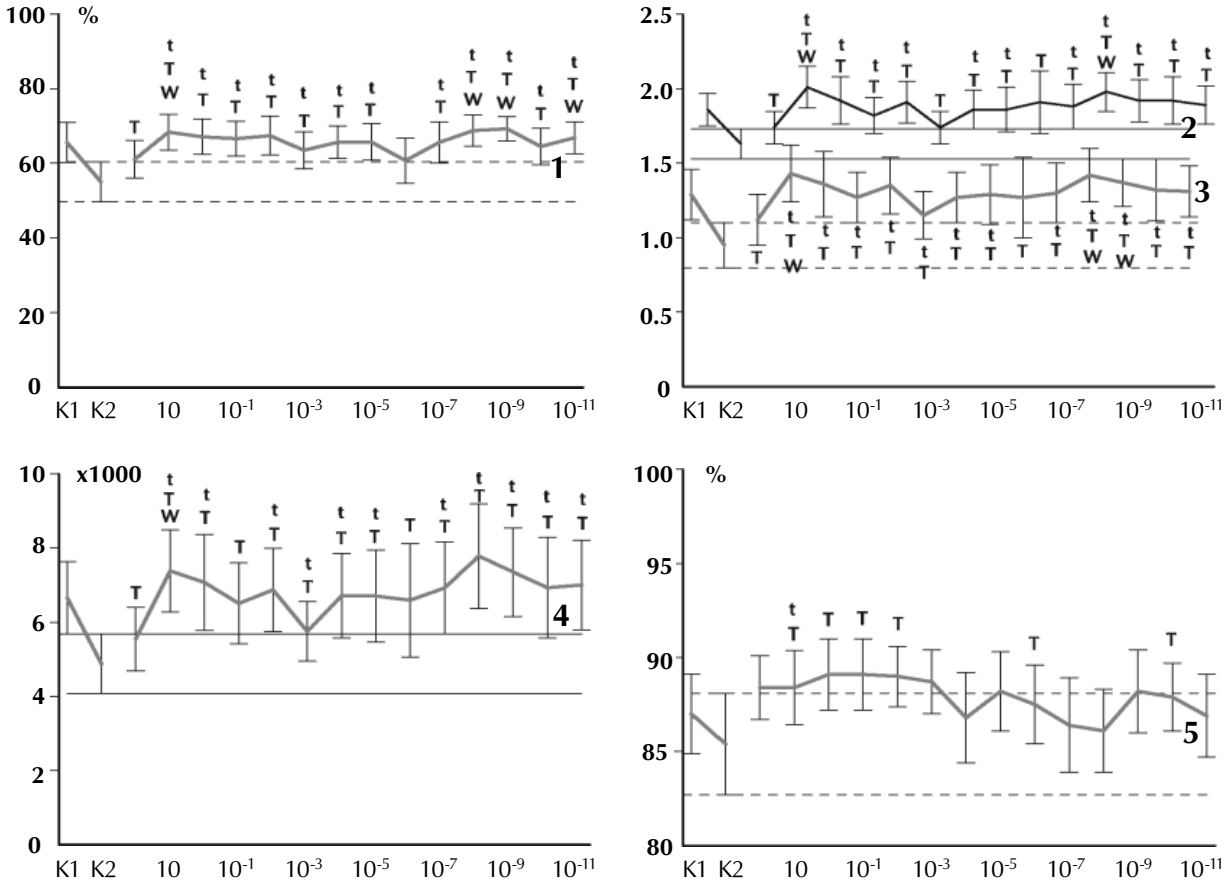


Figure 6. Influence of polyoxidonium on parameters of neutrophil phagocytic activity

1 - percentage of phagocytic neutrophils, **2** – phagocytic index, **3** – phagocytic number, **4** – absolute number of captured phagocytosis particles per 1 ml of blood, **5** – specific contribution to the total phagocytosis (M±m). **Axis X:** K1 – control parameters without preincubation, K2 – control parameters with cell preincubation in the medium, numerals denote final concentrations of drug (mg/ml). t – $p<0.05$ compared to control with preincubation (K2) using paired Student's criterion; T is the same using paired Wilcoxon's T-criterion; W is the same using unpaired Wilcoxon's criterion.

activity of peripheral blood leukocytes of the patient. For development and use of such tests, first of all, the exact information is needed dealing with dependence drug dose-effect on phagocytic activity of leukocytes. However in available literature such data are absent. Therefore the second task of the performed investigations was to study the dependence dose-effect of polyoxidonium influence on phagocytic activity of peripheral blood neutrophils, eosinophils and monocytes. It should be emphasized that to the present time the *in vivo* and *in vitro* drug influence on phagocytic activity of macrophages is more or less known in details in mice, as well as *in vitro* in concentration 100 μg/ml and *in vivo* on functional activity peripheral blood neutrophils in patients and healthy people [6]. Practically no studies were made pertinent to the action of polyoxidonium on phagocytic activity of human peripheral blood eosinophils and monocytes.

The results are given in **Figure 6-8**. The drug caused the most marked effect on neutrophil phagocytosis. The stimulating effect was revealed practically in all range of studied concentrations (Fig. 6). Statistically significant increase of percent of neutrophil contribution to the total leukocyte phagocytosis was shown (given parameter was calculated from absolute number of phagocytosis objects, ingested by phagocytes, contained in 1 μl of blood; therein the parameters of total leukocyte phagocytosis were taken for 100%; the detailed

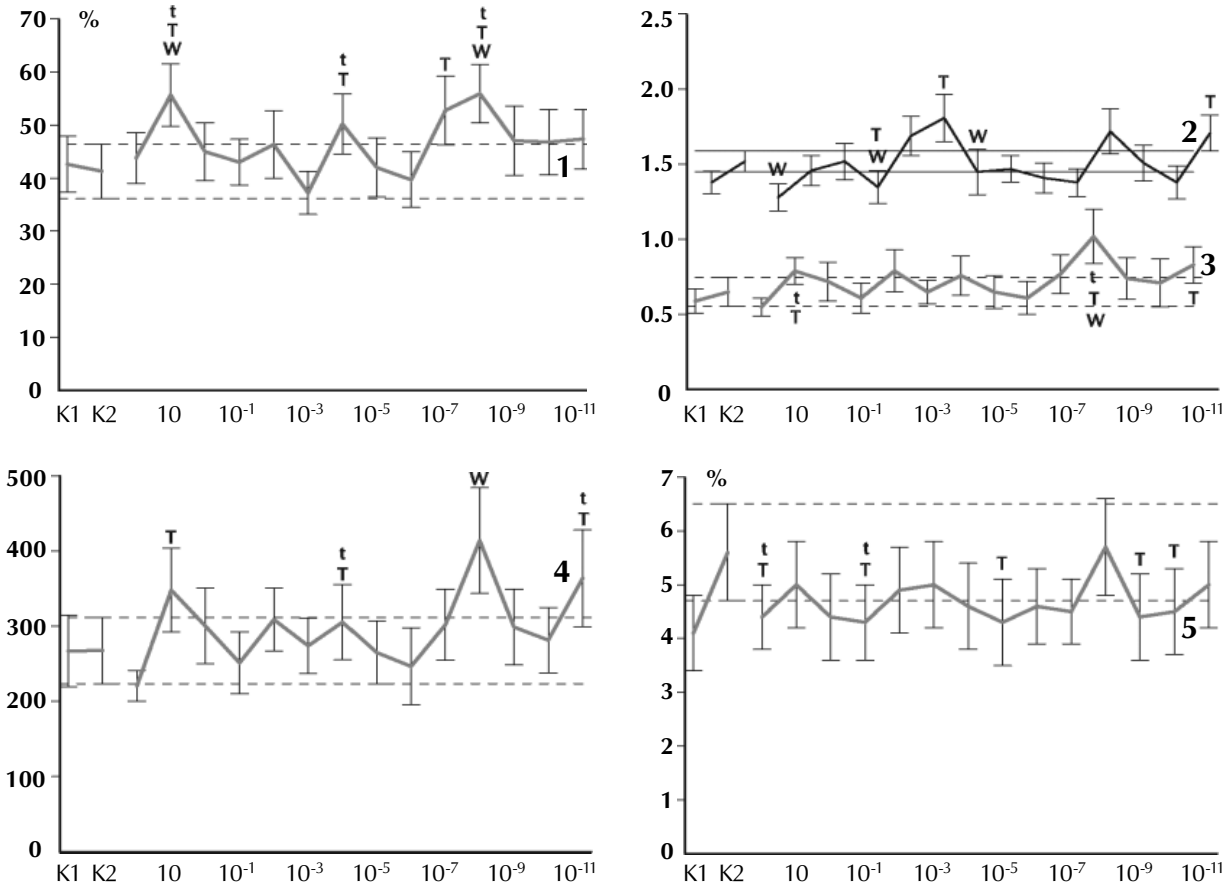


Figure 7. Influence of polyoxidonium on parameters of eosinophil phagocytic activity**.** The rest designations are the same that in Fig. 6.

technique of calculation was published earlier [22]). Polyoxidonium influence on eosinophil phagocytosis depended on its concentration (Fig. 7). The inhibition of phagocytic index was revealed in concentrations 100, 0.1 and 10^{-5} μg/ml, while in concentration 10^{-4} and 10^{-11} μg/ml the drug, on the average, increased the number of objects, ingested by one phagocytic eosinophil. As regards the other parameters of eosinophil phagocytosis (percent of phagocytic eosinophils, phagocytic number and absolute number of the ingested objects), the stimulation effect was prevailed. The relative specific eosinophil contribution was reduced due to the increase of neutrophil contribution in total phagocytosis. *In vitro* influence of polyoxidonium on phagocytosis of monocytes was much weaker and was seen only in concentrations 10^{-8} and 10^{-11} μg/ml (Fig. 8).

DISCUSSION

Immunomodulating Properties of Polyoxidonium

From animal models, according to parameters of the least degree of infiltration with immunocompetent of effector cells, scar structure, presence and character of commissures, and other signs, in injured eye, as well as even to the presence of minimal pathological changes in uninjured eyes, the most favorable course of wound process is pertinent to animals of the 3rd group received combined therapy in a complex with polyoxidonium.

Experiments *in vitro*, using peripheral blood cells from healthy donors, have shown that polyoxidonium mainly stimulates neutrophil and eosinophil phagocytosis. This effect is remained at minimal concentrations of drug. In final concentration 10^{-8} μg/ml polyoxidonium statistically significantly stimulates ingested activity of all blood

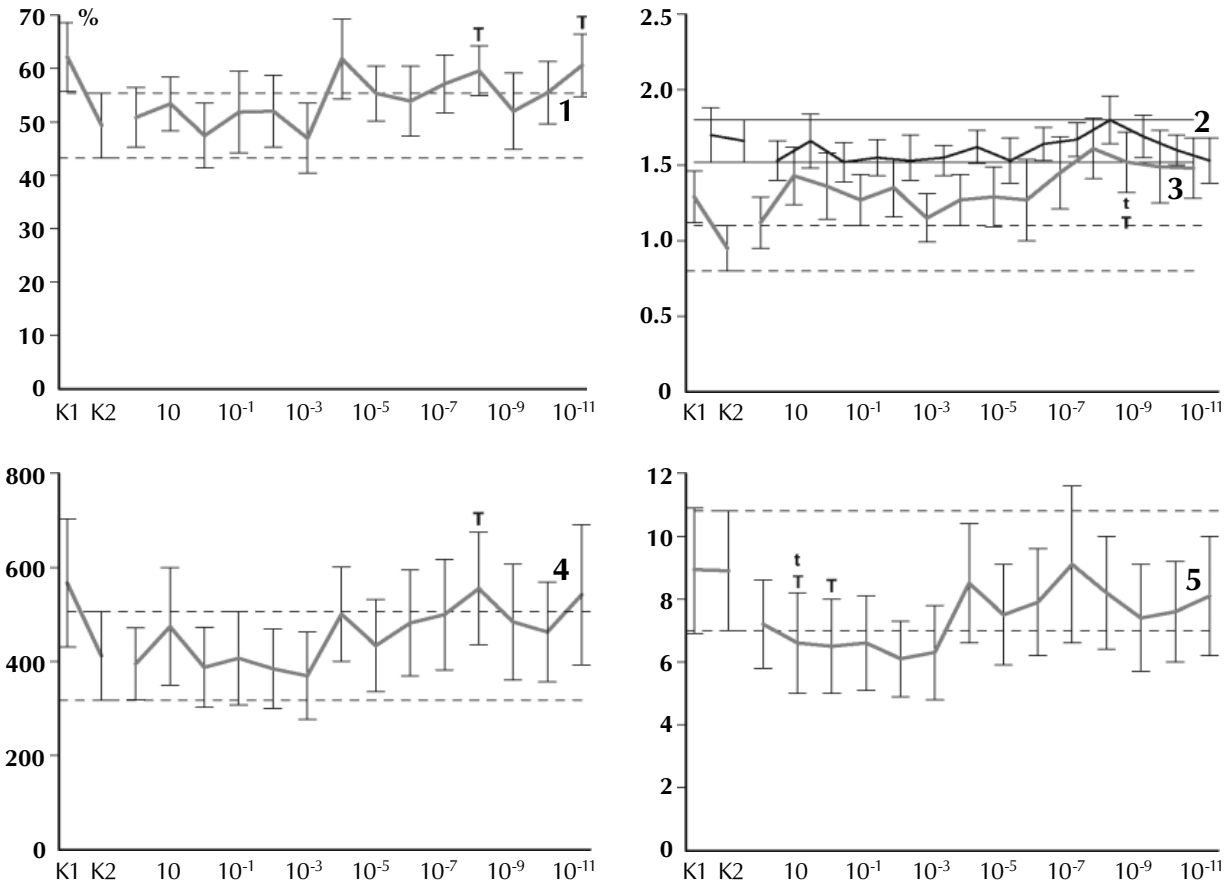


Figure 8. Influence of polyoxidonium on parameters of monocyte phagocytic activity. The rest designations are the same that in Fig. 6.

phagocytic cells, that allows to use this drug dilution for loading test. However in view of an opportunity that dependence dose-effect in patients may be changed, it is more reasonably to study the effect of a number of drug concentrations.

Influence of Complex Therapy with Addition of Polyoxidonium on Parameters of Immune Status in Patients with a Penetrating Eye Injuries

Our experience of polyoxidonium inclusion in the traditional protocols of treatment of severe penetrating eye wounds has shown the ambiguity of received results in dependence both on the character of injury, and on the features of the body response to it. It requires the individual approach to therapy. Some clinical cases are given below.

The patient K., 27 years old, has been admitted to clinic 18.11.98 with the diagnosis: penetrating infected wound of a cornea with iris prolapse, iridocyclitis, partial traumatic cataract of the right eye. A trauma was occurred 15.11.98, operation 18.11.98 - primary surgical procedure. Immune status was studied 23.11.98 on the background of standard therapy (oxacyllinum, gentamycin, indomethacin, dexamethasone subconjunctively from 18.11.98). There was revealed a decrease of parameters of NBT-test (absolute parameters of extinction per 120 μl of blood in stimulated variant amounted 0.579, and 0.436 in spontaneous one) without any changes of phagocytic activity of leukocytes (percent of phagocytosis - 65; phagocytic number - 1.03; phagocytic index - 1.59), T and B lymphocyte numbers, null-cells, concentration of IgG, IgA, IgM, as well as reduction of lymphocyte numbers increased CD2 affinity, inversion theophylline, adrenaline and T-activin tests. From

25.11.98. polyoxidonium was added (5 injections intramuscularly, 6 mg each according to special protocol), from 27.11.98 - dexamethasone (3 intravenous injections), from 02.12.98 - thymogen (intramuscularly according to protocol). 22.12.98 - acuity of vision was 0.7, eye is almost quiet, stitches are clean, partial cataract was remained; immune status: in comparison with the first immunogram - activation of parameters of NBT-test with inversion (stimulated variant -0.576, spontaneous one - 0.652), increase phagocytic leukocyte activity (percent of phagocytosis - 72; phagocytic number - 1.39; phagocytic index - 1.93), disappearance of inversion of theophylline, adrenaline and T-activin tests, normalization of relative and absolute lymphocyte numbers, increased CD2 affinity. However the absolute numbers of T and B lymphocytes were decreased almost in 2 times. Thus, the combination of positive dynamics may be seen, manifested both in clinical, and in immunological aspects.

The patient G., 16 years old, has been admitted to clinic 02.12.97 with the diagnosis: penetrating infected wound of a cornea with iris and vitreous humor prolapse, iridocyclitis, complete traumatic cataract of the left eye. A trauma was occurred 01.12.97, operation 02.12.97 - primary surgical procedure with suction of lens material, anterior vitrectomy. Study of immune status was made 04.12.97 on a background of standard therapy (ampicillin, gentamycin, indomethacin, dexamethasone subconjunctively from 02.12.97). There was revealed a decrease of separate parameters of phagocytic activity of peripheral blood leukocytes (percent of phagocytosis is 58.6, phagocytic number - 0.87; phagocytic index - 1.49) without any changes of NBT-test (absolute parameters of extinction in stimulated variant amounted 1.296, and 0.151 in spontaneous one), relative numbers of T lymphocytes (37%), inversion of theophylline and T-activin (but not adrenaline) tests, moderate increase of IgM concentration (2.62 g/l). From 05.01.98 polyoxidonium and thymogen intramuscularly during 1 month were added to the therapy. 09.02.98: acuity of vision - photoreception with a correct projection, eye with slight irritation, moderate manifestations of cyclitis are maintained, complete cataract; immune status: in comparison with the first immunogram - activation of parameters of phagocytosis (percent of phagocytosis - 63; phagocytic number - 1.43; phagocytic index - 2.27) and of spontaneous variant of NBT-test (1.377) on a background of inversion and reduction of oxidative potential in stimulated sample (0.612). Concentration of IgM (5.40 g/l) has considerably increased that may be an evidence of active inflammation. Relative number of T lymphocytes increased up to 51%, the inversion of theophylline and T-activin tests is remained with appearance of inversion of adrenaline test. Thus, despite of the improvement of parameters of phagocytic leukocyte activity, the deficiency of a T-limb of immunity is remained in patient that under presence of clinical signs of inflammation dictates the necessity of continuation of complex treatment.

At whole, obtained data indicate that further studies and usage of immunomodulator polyoxidonium, synthesized in the Institute of Immunology of Russian Federation Ministry of Health, are promising in complex therapy in penetrating eye injuries.

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