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CHANGES OF INDUCIBLE NO SYNTHASE AND INTERLEUKIN-1B ON THE BACKGROUND OF EXPERIMENTAL RHEGMATOGENOUS RETINAL DETACHMENT

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Abstract

Purpose: inflammatory processes investigation in the rhegmatogenous retinal detachment pathogenesis and analysis of correction methods effectiveness.

On the 7th day in experimental groups observed increase in the interleukin 1β level and inducible NOS activity. On 14th day of the research, as in the 7th, also confirms inflammatory process development.

In addition to presence of statistically significant differences between iNOS and IL 1β indices of all experimental groups in comparison with the data of intact animals at the level of significance p <0,05 (and iNOS - at the level of significance p <0,01), the tendency towards increased activation of inflammation in the group that did not receive corrective therapy, and decrease - in group № 4.

The most positive therapeutic effect was observed in the 4th group at 21st day. Compared with data of intact rats in group № 2, for given day, increased activity of e inducible NOS at the level of significance was already p <0.01. Differences of group № 3 remained at level of significance p <0,05. In this case, no differences were found when comparing inducible NO-synthase activity in animals of group number 4 and control group.

At the same time, there are very significant differences in the comparison of data groups № 4 and № 2. the superiority of the proposed correction suggests decrease in iNOS pathological activity in the rats of the above group compared with the data of the third group ($p < 0,05$). In the analysis IL 1β dynamics at the 21st day, there was no difference in the results of groups number 3 and number 4 in comparison with the values of intact animals. At the same time, difference between fourth group, which received the correction proposed by us in comparison with the data of the second group, in which the simulated pathology was not adjusted, increased. Foregoing confirms the effectiveness of cytokoline and D-asparagine use in complex with the administration of L-arginine 7% solution against the background of experimental rhegmatogenous retinal detachment.

Key words: rhegmatogenous retinal detachment, correction, interleukin 1β (IL 1β), inducible NO synthase (iNOS), cytokoline, D-asparagine, L-arginine.

Introduction. Analysis of the literature showed that rhegmatogenous retinal detachment (RRD) is one of the main blindness and visual impairment causes, since its treatment is not high enough. Despite the modern methods of diagnosis and treatment that allow receiving a positive anatomical result - retina adherence in almost 100% of patients - visual functions are often very low [1, 2].

According to the literature, it has been shown that retina damage occurs not only due to its detachment, but is greatly enhanced after surgical interventions [3, 4]. It is shown that repeated operations allow to improve the anatomical results of treatment, but the visual acuity remains lower than after the first operation [5].

Recently, researchers paid attention to determining role of inflammatory processes in the retinal detachment pathogenesis [6-8]. It is proved that local inflammation development plays significant role in RRD pathogenesis: in patients with RRDs, the concentration of interleukin 1β and interleukin 6 proinflammatory cytokines, ratio coefficient of pro- and anti-inflammatory cytokines and lactoferrin in lacrine and subretinal fluid is significantly increased [9].

Purpose: inflammatory processes investigation in the rhegmatogenous retinal detachment pathogenesis and analysis of correction methods effectiveness.

Materials and methods

In the study were used 120 white rats of the Wistar line. According to the tasks the animals were divided into 5 groups:

1st group – 20 intact animals; 2nd group – 36 animals, in which rhegmatogenous retinal detachment was modeled; 3rd group – 36 animals that received cytokoline and 0.1% of D-asparagine on the background of simulated retinal detachment. 4th group – 36 animals, received cytokoline and D-asparagine in combination with L-arginine 7% solution administration against background of simulated retinal detachment.

Rhegmatogenous retinal detachment was modeled using the introduction of 3.5 μl of sodium hyaluronate into the subretinal space. Administration was carried out using a self-sealing scleral incision (after the cut of the conjugate) using a needle 30 G, with further formation of a scleral tunnel for sclera and choroid penetration and a corneal puncture to reduce intraocular pressure. Sodium hyaluronate administration into subretinal space was performed with 33 G needle, connected with Hamilton syringe of 10 μl, to detach neurosensory retina from the underlying RPE (retinal pigment epithelium) (Matsumoto H., Miller J. W., Vavvas D. G., 2013 in combination with mechanical injury to the frontal part of the head (modeled traumatic brain injury (TBI) using Shubin's O.S. and Egorova MV method, 1999, reducing in twice standard weight (importance) of traumatic factor and the height of the fall. It was chosen weight 25 g and the height of its incidence of 50 cm, which provides less pronounced pathological changes in mild traumatic brain injury).At the 7th, 14th, and 21st days of the experiment were taken out 12 rats of each group in which rhegmatogenous retinal detachment was modeled.

It was conducted blood samples from retroorbital venous plexus, which lies in the orbit behind eyeball under light, ethereal anesthesia. Puncture is carried out by glass pipette circular movements with a drawn capillary, whose tip is stuck at an angle of 45°. Conjunctival sac was punctured in the medial angle of the eye between eyeball and orbit. After puncture, pipette was injected to 2-4 mm depth for eyeball. Control of penetration into the venous plexus was the filling of the capillary pipettes with blood (Dyakonov AV, Khrikina IS, Hegay AA, et al., 2013).

Dosage: Cytokoline - 81.8 mg/kg (0.33 ml/kg) intramuscularly for 14 days and once a day. D-asparagine - 0.1% solution. L-arginine - 7% solution. Solutions of D-asparagine and L-arginine were dissolved in 100 ml of water and given in a free drinking mode. Cytokoline, D-asparagine and L-arginine were administered from 7 day to 21 day from the beginning of the study.

Research results. In studying inflammatory process development in the conditions of our experiment there are tendencies that were identified in the analysis of all previous biochemical indicators. This is evidenced by interleukin-1β (IL 1β) results and inducible NO

synthase dynamics (Table 1-3). So, on the 7th day (Table 1) in experimental groups observed increase in the interleukin 1β level and inducible NOS activity. The increase is statistically significant in comparison with the data of intact animals at the level of significance p <0,05. At the same time, group № 2, 3, and 4 does not have differences in results.

Table 1. Dynamics of IL 1β and iNOS activity in rat blood in experimental rhegmatogenous retinal detachment and its correction for the 7th day of the study.

	Intact animals 1	7th day		
		Group №2 2	Group №3 3	Group №4 4
iNOS	0.18±0.01	0.23±0.01 p_{21}*	0.22±0.01 p_{31}*	0.23±0.01 p_{41}*
Iл 1β	29.4±1.0	34.0±0.8 p_{21}*	34.2±1.0 p_{31}*	34.1±0.9 p_{41}*

Footnote: * – p<0,05; ** – p<0,01

On 14th day of the research, as in the 7th, also confirms inflammatory process development (Table 2), as evidenced by the results of the Interleukin level determination 1β and inducible NO synthase.

Table 2. Dynamics of IL 1β and iNOS activity in rat blood in experimental rhegmatogenous retinal detachment and its correction for the 14th day of the study.

	Intact animals 1	14th day		
		Group №2 5	Group №3 6	Group №4 7
iNOS	0.18±0.01	0.25±0.01 p_{51}**	0.22±0.01 p_{61}*	0.20±0.01 p_{75}*
Iл 1β	29.4±1.0	35.1±1.1 p_{51}*	33.5±1.4 p_{61}*	32.0±1.0 p_{71}*

Footnote: *– p<0,05; ** – p<0,01

At the same time, besides the presence of statistically significant differences between iNOS and IL 1β indices of all experimental groups in comparison with the data of intact animals at the level of significance p <0,05 (and iNOS - at the level of significance p <0,01),

the tendency towards increased activation of inflammation in the group that did not receive corrective therapy, and decrease - in group № 4.

The most positive therapeutic effect was observed in the 4th group at 21st day (Table 3). Compared with data of intact rats in group № 2 (which did not receive corrective therapy), for given day, increased activity of e inducible NOS at the level of significance was already p <0.01 (on 14th day the differences were found at p <0.05). Differences of group № 3 remained at level of significance p <0,05. In this case, no differences were found when comparing inducible NO-synthase activity in animals of group number 4 and control group. At the same time, there are very significant differences in the comparison of data groups № 4 and № 2 in favor of the correction that was carried out in experimental animals of the fourth group. Also, the superiority of the proposed correction suggests decrease in iNOS pathological activity in the rats of the above group compared with the data of the third group (p <0,05).

On Fig. 1 reflects inducible nitric oxide synthase activity dynamics throughout the experiment. Traced its increase at each stage in the group, in which the modeled pathological process has not been corrected. Also, is clearly shown gradual normalization of this enzyme activity in groups with correction of the experimental RRD, which is more pronounced in the 4th group.

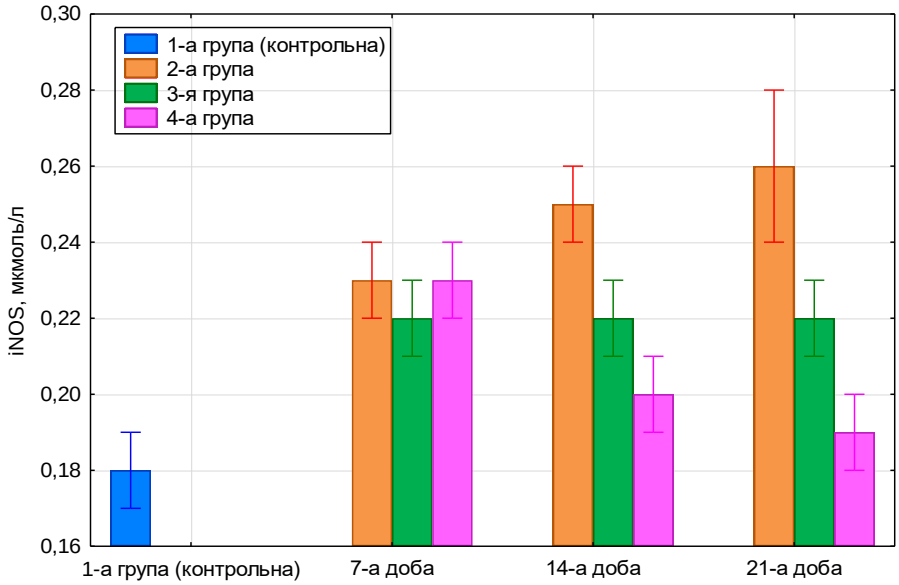

Figure 1 - Change inducible NO-synthase activity in experimental animals blood in the dynamics after the experimental rhegmatogenous retinal detachment and its correction.

In analyzing IL 1β dynamics, following is detected. Differences of second group in comparison with the control remained at the level of significance p <0,05. It was revealed differences absence in the results of groups № 3 and №4 in comparison with the values of intact animals. At the same time, difference between fourth group, which received the correction proposed by us in comparison with the data of the second group, in which the simulated pathology was not adjusted, increased. Foregoing confirms the effectiveness of cytokoline and D-asparagine use in complex with the administration of L-arginine 7% solution against the background of experimental rhegmatogenous retinal detachment.

Table 3. - Dynamics of IL 1β and iNOS activity in rat blood in experimental rhegmatogenous retinal detachment and its correction for the 21st day of the study.

	Intact animals 1	21st day		
		Group №2 8	Group №3 9	Group №4 10
iNOS	0.18±0.01	0.26±0.02 p_{81}**	0.22±0.01 p_{91}*	0.19±0.01 $p_{10\text{-}8}$**, $p_{10\text{-}9}$*
Iл 1β	29.4±1.0	35.0±1.0 p_{81}*	31.4±1.0	29.9±0.9 $p_{10\text{-}8}$**

Footnote: *– p<0,05; ** – p<0,01

Analyzing the results of proinflammatory cytokine, we observe inflammatory process development in all groups with simulated pathology (Figure 2).

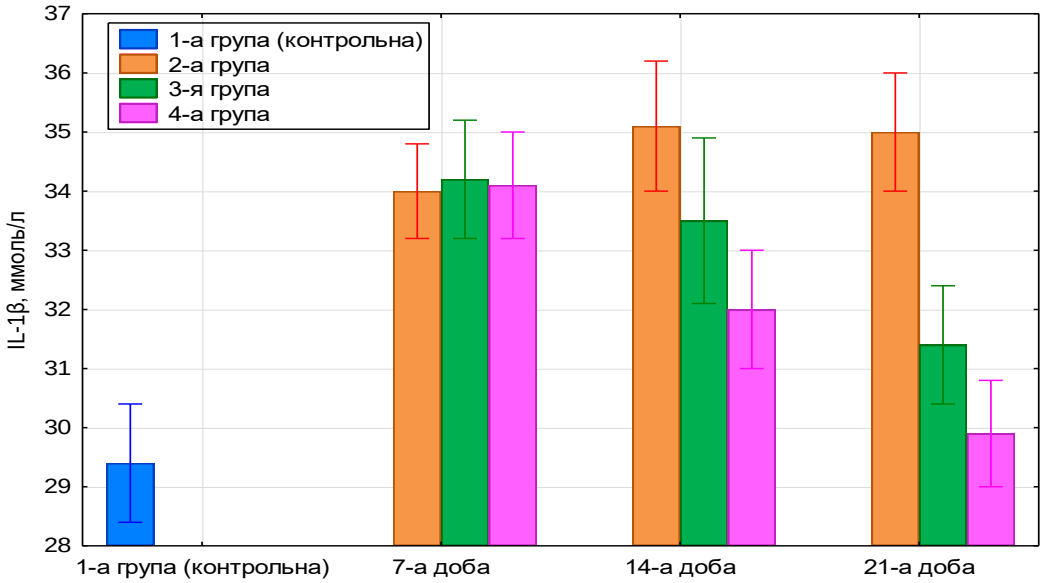

Figure 2. Change Interleukin -1ß content in the blood of experimental animals in the dynamics after experimental rhegmatogenous retinal detachment and its correction

In the second group there is Interleukin -1ß increase from the 7th to the 14th day of research; in interval between the 14th and the 21st day, level of this indicator in group 2 is constant. Both correction methods reduce the inflammatory process in experimental animals. In group № 4, more pronounced normalization of this indicator was detected.

Research results discussion. In our experimental research, it was found that in animals, which were modeled rhegmatogenous retinal detachment, level of Interleukin 1β was increased by 15,6% on the 7th day, by 19,4% on the 14th and by 19% on the 21st day in the blood of experimental animals, which wasn't corrected for an experimental pathological condition.

From literature sources, there is a relationship between inducible NO synthase and pro-inflammatory Interleukin 1ß. For more complete understanding of all pathogenetic links of the studied pathology, it was important for us to investigate the presence or absence of the aforementioned mutually reinforcing relationship of inducible NO synthase and Interleukin 1ß. As for inflammation, as noted, based on dynamics of Interleukin 1ß in the conditions of our pathology, we can state that it is activating In addition, inducible NO-synthase activity is increased on the background of the experimental RRD: by 27,8% on the 7th day, by 38,9% on the 14th and by 44,4% on the 21st day of the study compared with data of intact rats. And here we can observe the so-called vicious circle - the interconnection of endothelial dysfunction (activation of inducible nitric oxide synthase is associated with endothelial inhibition) with inflammation in the rhegmatogenous retinal detachment. Therefore, choosing pathogenic justified correction, it is necessary to take into account also this connection, which arises at RRD and correct it. We have found the following trend regarding the correction efficiency: combination of L-arginine with cytokoline and D-asparagine (Group 4) is more effective. So in group №3 for the 14th day, the level of proinflammatory cytokine was reduced by 4,6%, and in the fourth group - by 8,8% compared with the results of group №2. Activity of inducible nitric oxide synthase at this stage in the third group decreased by 12%, and in group № 4 - by 20% compared with the data of animals for which the pathological process was modeled without correction.

Arginine has anti-inflammatory properties, as well as an approximation of inducible NO-synthase activity to the values of intact animals. This is also confirmed by the data obtained at the 21st day of the study: in group 3, the level of interleukin 1β was decreased by 15,4%, the activity of inducible NO synthase was reduced by 10,3% compared with the group, which was not corrected. At the same time, in the group receiving a three-component

correction, reduction of the studied indicators compared with the data of group number 2 is set at 26,9% and 14,6% respectively.

Conclusions:

1. Increase in Interleukin-1β production by 19.0% in the modeled rhegmatogenous retinal detachment pathogenesis, indicating the involvement of the inflammatory process.

2. There is an increase in inducible nitric oxide synthase activity by 44.4% in the blood of experimental animals against the background of experimental rhegmatogenous retinal detachment.

3. In both groups, animals which were corrected pathological process, was revealed tendency towards normalization of the studied indicators.

4. In group 4, animals of which receiving cytokoline, D-asparagine and L-arginine showed more pronounced positive results, indicating significant reduction in the inflammatory process and decrease in the activity of inducible NO synthase ($p < 0.01$ at 21 day compared to group data without correction).

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