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DYNAMICS OF VASOCONSTRICTION AND VASODILATION POTENTIAL ON THE BACKGROUND OF EXPERIMENTAL RHEGMATOGENOUS RETINAL DETACHMENT AND ITS CORRECTION

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Abstract

Purpose: investigation of vasoconstriction and vasodilation potential in rhegmatogenous retinal detachment pathogenesis and analysis of correction methods effectiveness. It's proven that endothelial dysfunction is an important link in the pathogenesis of experimental rhegmatogenous retinal detachment. Balance violation of the vasoconstrictor and vasodilator potential in the direction of vasoconstriction predominance mechanisms against the background of the studied pathology is established.On the background of experimental rhegmatogenous retinal detachment found increased endothelin-1 level by 16.1%, and S-nitrosothiols level decreased by 26.3% in the blood of the experimental animals. The most characteristic changes were detected at the 21st day ($p < 0.01$ compared to intact rats). In both groups, which corrected the experimental rhegmatogenous retinal detachment, was revealed tendency toward restoration of the physiological balance of the vasoconstrictor and vasodilator potential. More pronounced correction efficiency is established in group in which the animals received cytokoline, D-asparagine and L-arginine against the background of the modeled rhegmatogenous retinal detachment.

Key words: experimental rhegmatogenous retinal detachment, correction, citokoline, D-asparagine, L-arginine.

Introduction. Rhegmatogenous retinal detachment (RRD) occupies important place among disability and blindness causes, which determines the medical and social significance of this problem and causes the need for further pathogenesis study and new effective methods development of RRD correction.

This pathology occurs among adult Ukraine population with frequency of 3 cases per year per 10,000 people, increasing from 1 to 10,000 people aged 20, up to 7 times making diagnoses per 10,000 people after 70 years [1].

RRD – photoreceptors detachment from retinal pigment epithelial by retrovitreal fluid, which penetrates through the breakdown of the neurosensory part.

Unfortunately, surgical intervention at rhegmatogenous retinal detachment in many cases doesn't update functional eye indicators results, which may remain low. Retina adherence, achieved surgically, in most patients leads only to partial restoration of functional and structural retina elements, with least restored cone apparatus, which is confirmed by electroretinography data [2].

Ratio violation against the backdrop of retinal detachment, macular area and photoreceptor layers are most likely suffer from oxygen lack and metabolic substrates in comparison with its internal layers, although they also undergo significant degenerative changes.

Key link in RRD pathogenesis is hypoxia. This is due to the fact that retina is more intense consumes oxygen than other tissues, it is characterized by enzymes glycolysis high activity, main Krebs energy cycle and oxidative phosphorylation, so it is very sensitive to hypoxia, as well as to oxidation substrates lack. At certain stage of ischemia and hypoxia, neurons damage becomes irreversible, therefore the retinal detachment can be considered as "ischemic illness of the eye".

Purpose: investigation of vasoconstriction and vasodilation potential in 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.

Content of S-nitrosothiols as known, are stable NO metabolites, were determined by spectrofluorimetric method. Endothelin-1 content determination was carried out by immunoassay in serum. Determination of vasoconstrictor or vasodilation potential dominance

index was performed by determining the ratio of endothelin-1 / S-NO. The need for this unit of research is due to well-known from literary sources data about blood flow deterioration in patients with rhegmatogenous retinal detachment, which may lead to increase in the number of peripheral vitreous chorioretinal dystrophy foci (N.G. Zavgorodnya, A.V. Dedov, A. M. Ivakhnenko, 2014).

Since the ranks of the measured index in all samples did not differ from the normal statistical distribution, which was checked by the Shapiro–Wilk test, which is most sensitive to relatively small sample sizes, further mathematical and statistical processing of research results was conducted using descriptive statistics (M ± m) where M is arithmetic mean, m is the standard error. Intergroup comparisons were carried out using Student's t test.

Research results and their discussion

Research results of S-nitrosothiols (S-NO) content .

On the seventh day, decrease in the S-nitrosothiols content was found in all groups in which the animals were modeled by rhegmatogenous retinal detachment. Differences between these groups in comparison of data have not been established (Table 1).

Table 1. S-NO content dynamics in rat blood at experimental rhegmatogenous retinal detachment and its correction

		Groups with modeled detachment		
S-NO	Intact animals 1	Group №2 2	Group №3 3	Group №4 4
7th day	0.38±0.01	0.34±0.01 p_{21}*	0.35±0.01 p_{31}*	0.33±0.01 p_{41}*
14th day	0.38±0.01	0.30±0.02 p_{21}**	0.35±0.02 p_{31}*	0.36±0.01 p_{41}* p_{42}*
21st day	0.38±0.01	0.28±0.01 p_{21}**	0.36±0.01 p_{31}*	0.39±0.03 $p_{4\text{-}1}$*

Footnote: * – p<0,05

On the 14th day in the group that didn't receive corrective therapy in restoring rhegmatogenous retinal detachment, under experiment revealed significant vasodilation potential deterioration (differences at the significance level of $p < 0.01$ as compared with the data of the same group in the previous research stage, and with results of intact animals). In the group receiving the 3-component correction (group 4), an increase in S-nitrosothiols content was observed compared to the data of group 2 ($p < 0.05$) (Table 1).

At 21st day, attract attention presence of differences at the level of significance p <0.05 when comparing the results of 4th group S-NO content results with all groups at each research stage. Obtained data indicate that the 3-component correction proposed by us effectively restores vasodilatation potential of vessels, which most expressively manifests itself at the 21st day of the study (14th day of drug administration) (Table 1).

Endothelin-1 reserch results

At day 7, was found increase in this vasoconstriction marker in all groups in which rhegmatogenous retinal detachment was modeled (p <0.05 when compared with control group). When comparing these groups between them, their homogeneity is confirmed (Table 2).

Table 2. Endothelin-1 level dynamics in rats blood at experimental rhegmatogenous retinal detachment and its correction

	Intact animals 1	Groups with modeled detachment		
		Group №2 2	Group №3 3	Group №4 4
7th day	3.30±0.11	3.61±0.08 p_{21}*	3.63±0.08 p_{31}*	3.60±0.07 p_{41}*
14th day	3.30±0.11	3.78±0.09 p_{21}**	3.63±0.07 p_{31}*	3.64±0.08
21st day	3.30±0.11	3.83±0.09 p_{21}**	3.55±0.06	3.50±0.08

Footnote * – p<0,05, ** – p<0,01

On the 14th day, was found more pronounced vasoconstrictor potentials progression in group of animals, which pathological state under research was modeled without further correction. In the group in which the experimental rhegmatogenous retinal detachment was corrected using cytokoline, D-asparagine and L-arginine there were no differences when compared with the data of rats in the control group.

This demonstrates effectiveness of proposed treatment to improve the functional state of the endothelium in the context of weakening pathologically increased vasoconstrictor potential (Table 2).

Correction efficacy, which was received by 4th group of the researched rats, is found improve on the 21st day of the research. In the group that did not receive any corrective measures, progression of vasoconstriction was established (as evidenced by an increase in the

endothelin-1 content at the level of significance p <0.01 compared with the control group data) (Table 2).

Research result discussion

Also, for a more complete understanding of rhegmatogenous retinal detachment pathogenesis we conducted a study of vasoconstrictor and vasodilatation potential of vessels. Again, we return to the already noted fact about the retina sensitivity to hypoxia, to circulatory disorder. And what is important - that pathological process correction improve the blood supply of damaged animals, increase access to damaged tissue corrective substances when correcting the pathological process, and also to improve the blood supply itself of these tissues and cells in order to better and faster restore their functions and reduce the pathological load. So for this purpose, we examined endothelin-1level, which isn't only endothelial dysfunction marker, but also is vasoconstriction indicator. Content of S-nitrosothiols, which is a marker of vasodilation was investigated. Endothelin-1, which is also a pronounced vasoconstrictor, whose activity is 10 times greater than angiotensin II, and 100-fold the effect of norepinephrine, is the most representative of the endothelin family [3].

During investigation, an increase in the endothelin-1 (E-1) content, one of the main endothelial dysfunction markers [4], was observed on the background of experimental rhegmatogenous retinal detachment Concentration of endothelin-1 in blood is a key marker for the implementation of the vasoconstrictor or vasodilatory effect [3]. Its increase suggests imbalance strengthening between the vasodilatory and vasoconstriction systems of vascular regulation in favor of the latter [5]. On the seventh day in animals, which were modeled experimental rhegmatogenous retinal detachment without further correction revealed an increase in the level of E-1 by 9.4%. On the fourteenth day the content of this indicator was increased in the second group by 14.5%, and at the 21st - by 16.1%.

At low concentrations, endothelin acts on endothelial cells, contributing to vasodilatory effects, and with increased concentrations leads to vascular spasm [6]. Currently, direct and non-direct effects of endothelin are known. Direct effect is a direct effect on the smooth muscles of the vessels. During this action, cell proliferation, vasoconstriction, intima fibrosis, increased rigidity of blood vessels and activation of lithogenesis occur. Indirect effect occurs in the release of nitric oxide, prostacyclin and atrial natriuretic peptide from the endothelium, which leads to vessels relaxation [3]. It is generally accepted to consider endothelin-1 as a diagnostic marker for severe cardiovascular pathologies [7, 8].

And in groups, which were corrected, there is decrease in pathologically increased vasoconstrictor potential. At 14th days, was found decrease in the level of endothelin-1 in

comparison with the data 2nd group by 4% in the third, and by 3.7% in the fourth group. On the 21st day - by 7.3% and 8.6% respectively.

In research of S-nitrosothiols content, we have established weakening of the vasodilation potential by 10.5% in the group, which was not corrected by the simulated RRD. At day 14 in this group, the level of S-NO was reduced by 21.1%, at the 21st - by 26.3%. Corrective therapy has proved its effectiveness in restoring vasodilation potential: at the 14th day in the third group, the level of the indicator increased by 16.7%, and in the fourth - by 20% compared with the results of animals in group number 2. On the 21st day, an improvement was set at 28.6% and 39.3%, respectively, in the third and fourth groups.

It should be noted that the more pronounced positive effect gave complex combination of all three proposed drugs (Group № 4), and most significant - at the 21st day of the resesarch, indicating that the more accumulation of the drug in the body, the better its therapeutic effect. That is, L-arginine was well manifested in the studied pathology. This can be explained by the fact that L-arginine prevents oxidation of the main co-factor of the eNOS [9]. Also, this amino acid prevents inhibition of endothelial nitric oxide synthase competing with asymmetric dimethyl L-arginine [10].

It is also worth noting that the pathological component reduction of vasoconstriction is definitely important component of the pathogenetically conditioned correction of rhegmatogenous retinal detachment, since it is aimed at improving the blood supply to the retina, and accordingly, and the restoration of functioning.

Conclusions:

1. It’s proven that endothelial dysfunction is an important link in the pathogenesis of experimental rhegmatogenous retinal detachment.

2. Balance violation of the vasoconstrictor and vasodilator potential in the direction of vasoconstriction predominance mechanisms against the background of the studied pathology is established.

3. On the background of experimental rhegmatogenous retinal detachment found increased endothelin-1 level by 16.1%, and S-nitrosothiols level decreased by 26.3% in the blood of the experimental animals. The most characteristic changes were detected at the 21st day ($p < 0.01$ compared to intact rats).

4. In both groups, which corrected the experimental rhegmatogenous retinal detachment, was revealed tendency toward restoration of the physiological balance of the vasoconstrictor and vasodilator potential.

5. More pronounced correction efficiency is established in group in which the animals received cytokoline, D-asparagine and L-arginine against the background of the modeled rhegmatogenous retinal detachment.

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