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THE STUDY OF AMINO ACIDS LEVEL IN THE VITREOUS BODY OF EXPERIMENTAL ANIMALS IN REGAMATOGENOUS RETINAL DETACHMENT

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ABSTRACT

INTRODUCTION: The paper presents the results of the amino acid levels study in the vitreous body of rats with rhegmatogenous retinal detachment (RRD).

MATERIAL AND METHODS: Experimental studies were performed on 42 brown Norwegian male rats (Male Brown Norway), which were divided into 7 groups (6 animals in each group): 1 group — conditionally intact control (without retinal detachment) — animals that underwent paracentesis of the anterior chamber with the removal of its moisture and retinal puncture without the introduction of any substance under the retina; 2 group — rats, which reproduced RRD by the method (control pathology) based on the study of apoptosis induction by apoptosis-inducing factor.

RESULTS: In the group of conditionally intact control animals the concentration of amino acids in vitreous body were: alanine — 3.3 ± 0.27 ng/mL; arginine — 0.8 ± 0.1 ng/mL; aspartate — 2.3 ± 0.5 ng/ /mL; valine — 1.8 ± 0.8 ng/mL; histidine — 1.3 ± 0.3 ng/mL; glycine — 4.5 ± 0.8 ng/mL; glutamic acid — 1.56 ± 0.35 ng/mL; tyrosine — 0.084 ± 0.048 ng/mL; phenylalanine — 0.024 ± 0.017 ng/mL; methionine — 0.11 ± 0.1 ng/mL. In animals with model pathology of RRD was observed a significant increase in certain amino acids: the level of alanine increased 1.4 times, aspartate — 11.5 times ($p < 0.05$), glycine — 2.3 times ($p < 0.05$), glutamic acid — 7.9 times ($p < 0.05$) compared with rats of the conditionally intact group. Levels of other amino acids increased insignificantly.

CONCLUSIONS: The most pronounced therapeutic efficacy was found in rats treated with combination therapy with dexamethasone, resveratrol, erythropoietin, and edaravon for 7 days. The pronounced effect of combination therapy on the level of amino acids (aspartate, glutamate, alanine, and glycine) in the treatment of RRD is due to the pharmacological activity of the components of this therapeutic regimen and characterized by synergistic effects of each component in the key links of disease pathogenesis.

KEY WORDS: rhegmatogenous retinal detachment; amino acids; vitreous body; combination therapy

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INTRODUCTION

The retina is the inner lining of the eye, located between the choroid and the vitreous body (VB). The retina has the ability to perceive light because of the operation of a complex photoreceptor apparatus [1].

Rhegmatogenous retinal detachment (RRD) occurs against the background of penetrating retinal rupture, resulting in fluid from the VB enters to the retina [2]. The main place among the etiological factors leading to the development of RRD is occupied by peripheral vitreochorioretinal dystrophies on the background of myopia and sclerotic dystrophy, VB pathology, eye injuries, etc. Despite the rapid development and achievements of modern ophthalmic surgery, the problem of restoring vision in retinal detachment is still one of the main causes of blindness and disability, because the prevalence of this disease is from 8.9 to 24.4 cases per 100 000 people per year, up to 30% of cases — with bilateral lesions [1, 3].

Recently, the study of retinal neurochemical activity (in particular, determination of amino acid levels) in normal and in pathological conditions, is of particular interest because these compounds are involved in neurotransmitter, metabolic processes, osmotic regulation, and protein synthesis. They also play an important role in the pathogenesis of retinal degeneration, glaucoma, diabetic retinopathy, retinitis pigmentosa, RRD, and other retinal injuries [4].

It is known that as a result of RRD there are significant neurochemical changes that develop from several days to several weeks. The study of the distribution of amino acids-neurotransmitters: glutamate, glycine, metabolic amino acids aspartate, and glutamine in experimental retinal detachment showed that in this case there are changes in the glutamatergic system of the neural retina, causing a massive release of neuronal glutamate changes [4–6].

The literature data were confirmed experimentally in a clinical study of the amino acid profile of VB and vitreal content in patients with different clinical characteristics of RRD [3, 7].

The study of amino acid levels of VB in rats with experimental modeling of RRD can supplement existing data on the pathogenesis of this disease and contribute to the correct choice of appropriate pathogenetic therapy. Therefore, the aim of the study was to establish the changes in amino acid levels in the VB rats under experimental RRD and with therapy.

MATERIAL AND METHODS

Experimental studies were performed on 42 brown Norwegian male rats (Male Brown Norway), which were divided into 7 groups (6 animals in each group): 1 group — conditionally intact control (without retinal detachment) — animals that underwent paracentesis of the anterior chamber with the removal of its moisture and retinal puncture without introducing any substance under the retina; 2 group — rats, which reproduced RRD by the method [8] (control pathology) based on the study of apoptosis induction by apoptosis-inducing factor. After puncture of the anterior chamber through the corneal limb for reducing intraocular pressure, approximately half of the superonasal-lower temporal neurosensory retina was separated by subretinal injection of 1% sodium hyaluronate into the subretinal space; groups 3–7 — animals, which reproduced the simulated pathology and then treated: rats of group 3 received dexamethasone (intramuscularly at a dose of 0.2 mL of solution at a rate of 1 mg/kg body weight of the animal); group 4 — dexamethasone in combination with erythropoietin (intraperitoneal suberitrostimulating dose of 50 IU/kg body weight); group 5 — dexamethasone in combination with edaravon (intraperitoneally at a dose of 10 mg/kg body weight); group 6 — dexamethasone in combination with natural bioflavonoid isolated from grapes and grape seeds — resveratrol (intragastric dose of 1500 mg/kg body weight); group 7 — dexamethasone in combination with erythropoietin, edaravon and resveratrol.

Doses of the studied drugs were administered once a day in recalculation, considering the generally accepted in experimental pharmacology coefficients of species resistance of Yu. R. Rybolovlev [9]. We removed rats from the experiment on day 7 after completion of the course of the injections [10].

To achieve the aim of our work, we analyzed the level of the following amino acids: alanine, arginine, aspartate, valine, histidine, glutamic acid, glycine, phenylalanine, tyrosine, and methionine. The amino acid profile was studied in the vitreous body of the eye. The samples of vitreous body were taken in the cold immediately after decapitation and placed in nitrogen liquid. Before the test providing all samples were stored in a refrigerator at the temperature regime –70°C.

Analysis of the amino acid composition was performed on an amino acid analyzer, model 835 High-Speed Amino Acid Analyzer (Hitachi, Ltd., Japan) on a column of 2.6 x 250. Detection of amino acids was

performed at 570 nm, except for proline (which was determined at 440 nm). The results were calculated by applying the formula using standard aminogram data, which was used to determine the content of specific amino acid and expressed in ng/mL [11].

During the work with animals, we considered the International Code of Medical Ethics (Venice, 1983), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and the General Ethical Principles for Animal Experiments adopted by the First National Congress of Bioethics (Kyiv, 2001), Directive 2010/63/EU of the European Parliament and Council on protecting animals used for scientific purposes, the Law of Ukraine "On protection of animals from cruel treatment" No. 440-IX of 14 January 2020 [12].

Statistical processing of the obtained results was performed using the program "Statistica 8.0". The probability of differences between the indicators of the control and experimental groups was determined by Student's test.

RESULTS

In the group of conditionally intact control animals the concentration of amino acids in vitreous body were: alanine — 3.3 ± 0.27 ng/mL; arginine — 0.8 ± 0.1 ng/mL; aspartate — 2.3 ± 0.5 ng/mL; valine — 1.8 ± 0.8 ng/mL; histidine — 1.3 ± 0.3 ng/mL; glycine — 4.5 ± 0.8 ng/mL; glutamic acid — 1.56 ± 0.35 ng/mL; tyrosine — 0.084 ± 0.048 ng/mL; phenylalanine — 0.024 ± 0.017 ng/mL; methionine — 0.11 ± 0.1 ng/mL (Tab. 1).

In animals with model pathology of RRD was observed a significant increase in certain amino acids: the level of alanine increased 1.4 times, aspartate — 11.5 times ($p < 0.05$), glycine — 2.3 times ($p < 0.05$), glutamic acid — 7.9 times ($p < 0,05$) compared with rats of the conditionally intact group. Levels of other amino acids increased insignificantly.

The results of the experimental study of amino acid changes in rats with modeling pathology during therapy are presented in Tables 2 and 3.

In a study of the valine, histidine, tyrosine, phenylalanine, and methionine levels, no significant difference was found between the group of intact control, control pathology and groups of rats treated with both mono- and combination therapy, which may indicate that these amino acids do not participate in the pathogenesis of RRD (Tab. 2).

Table 1. The level of amino acids in the vitreous body in rats with model pathology on the 7th day of experiment (X ± SX, n = 6)

Indicators, ng/mL	Intact group (n = 6)	Control pathology (n = 6)
Tyrosine, ng/mL	0.084 ± 0.048	0.092 ± 0.052
Phelylalanine, ng/mL	0.024 ± 0.017	0.035 ± 0.018
Methionine, ng/mL	0.11 ± 0.1	0.13 ± 0.12
Valine, ng/mL	1.8 ± 0.8	2.2 ± 0.36
Histidine, ng/mL	1.3 ± 0.3	1.5 ± 0.35
Alanine, ng/mL	3.3 ± 0.27	4.5 ± 0.35*
Arginine, ng/mL	0.8 ± 0.1	0.9 ± 0.12
Aspartate, ng/mL	2.3 ± 0.5	26.4 ± 2.4*
Glycine, ng/mL	4.5 ± 0.8	10.5 ± 1.8*
Glutamic acid, ng/mL	1.56 ± 0.35	12.4 ± 2.8*

* — $p < 0.05$ compared with the intact group of animals

Table 2. The level of valine, histidine, tyrosine, phenylalanine, methionine in the vitreous body of rats on the 7th day of the experiment (X ± S_X, n = 6)

Experimental group	Valine, ng/mL	Histidine, ng/mL	Tyrosine, ng/mL	Phelylalanine, ng/mL	Methionine, ng/mL
Intact group	1.8 ± 0.8	1.3 ± 0.3	0.084 ± 0.048	0.024 ± 0.017	0.11 ± 0.1
Control pathology	2.2 ± 0.36	1.5 ± 0.35	0.092 ± 0.052	0.035 ± 0.018	0.13 ± 0.12
Dexamethasone	2.1 ± 0.4	1.3 ± 0.4	0.086 ± 0.050	0.036 ± 0.017	0.11 ± 0.11
Dexamethasone + erythropoietin	2.1 ± 0.51	1.5 ± 0.41	0.085 ± 0.047	0.032 ± 0.016	0.10 ± 0.12
Dexamethasone + edaravon	1.9 ± 0.65	1.3 ± 0.45	0.088 ± 0.051	0.028 ± 0.016	0.12 ± 0.15
Dexamethasone + resveratrol	2.2 ± 0.42	1.4 ± 0.38	0.091 ± 0.047	0.002 ± 0.015	0.09 ± 0.15
Dexamethasone + erythropoietin + edaravon + resveratrol	1.7 ± 0.6	1.2 ± 0.25	0.090 ± 0.047	0.020 ± 0.015	0.13 ± 0.01

n — number of animals in each group

Table 3. The level of alanine, arginine, aspartate, glycine, glutamic acid in the vitreous body of rats on the 7th day of the experiment (X ± S_X, n = 6)

Experimental group	Alanine, ng/mL	Arginine, ng/mL	Aspartate, ng/mL	Glycine, ng/mL	Glutamic acid, ng/mL
Intact group	3.3 ± 0.27	0.8 ± 0.1	2.3 ± 0.5	4.5 ± 0.8	1.56 ± 0.35
Control pathology	4.5 ± 0.35*	0.9 ± 0.12	26.4 ± 2.4*	10.5 ± 1.8*	12.4 ± 2.8*
Dexamethasone	4.1 ± 0.32	0.75 ± 0.3	17.7 ± 1.8*/**	9.1 ± 1.3*	11.7 ± 2.1*
Dexamethasone + erythro-poietin	3.92 ± 0.38	0.87 ± 0.21	17.1 ± 1.7*/**	8.8 ± 1.3*	10.8 ± 2.1*
Dexamethasone + edaravon	3.41 ± 0.23*	0.85 ± 0.11	14.3 ± 1.7*/**	7.8 ± 1.1*	5.6 ± 0.82*/**
Dexamethasone + resveratrol	4.0 ± 0.38	0.69 ± 0.3	17.6 ± 1.8*/**	9.3 ± 1.3*	11.2 ± 2.1*
Dexamethasone + erythropoietin + edaravon + resveratrol	3.0 ± 0.25**	0.74 ± 0.14	7.2 ± 1.1*/**	4.8 ± 0.7**	4.9 ± 0.8*/**

* — $p < 0.05$ compared with the intact group of animals; ** — $p < 0.05$ compared with the control group of animals; n — the number of animals in each group (n = 6)

It was found that in groups of rats treated with dexamethasone; dexamethasone in combination with resveratrol; dexamethasone and erythropoietin did not lead to statistically significant changes in the levels of alanine, arginine, glycine, and glutamic acid compared with the group of untreated animals (Tab. 3).

Combination therapy led to a significant decrease in the level of aspartate 1.49 times ($p < 0.05$), 1.51 times ($p < 0.05$), and 1.54 times ($p < 0.05$) compared to the group of rats with simulated pathology, respectively.

DISCUSSION

The obtained data can be explained as a fact that as a result of retinal detachment there are significant neurochemical changes: the level of glutamate, glycine, alanine, aspartate increases due to changes in the glutamatergic system of the neural retina, which causes a massive release of neuronal glutamate and causes comorbid changes in its metabolism. The release of neuronal glutamate causes excitotoxicity and initiates structural changes [5].

The accumulation of glutamate leads to excitotoxic effects by increasing the stimulation of its receptor, increasing the level of intracellular calcium, and initiating a cascade of changes that will lead to apoptosis or necrosis [4, 13].

Accumulation of glutamate and aspartate in the vitreous body of rats with RRD may be as a result of their release from dead retinal ganglion cells, which leads to further damage of neurons [14]. Increased excitotoxicity of glutamate and aspartate in the vitreous body is associated with ischemic processes in the optic nerve [6]. The increase in glycine levels may be due to retinal ischemia caused by RRD in rats [15]. Our results in modeling of RRD in rats correlate with data obtained by other researchers [4–6, 15].

Changes in the level of aspartate when using dexamethasone therapy (in its effective dose) can be explained by its protective effect and indirect effects on total protein and albumin in VB, leading to more effective restoration of the permeability of the blood-brain barrier [15, 16].

The therapeutic effect of using the dexamethasone + resveratrol treatment can be achieved due to the synergistic effect of the components of the therapy — a natural antioxidant — resveratrol reduces the harmful effects of reactive oxygen species in RRD and inhibits the course of disease [17].

In the group of animals receiving combination therapy with dexamethasone and erythropoietin, changes in aspartate levels can be explained by the direct effect of dexamethasone on the course of RRD, while erythropoietin will play the role of cytoprotector and protect retinal photoreceptors, retinal detachment from local damage factors.

The use of combination therapy with dexamethasone and edaravon led to a decrease in the level of alanine 1.3 times ($p < 0.05$), aspartate — 1.8 times ($p < 0.05$); glutamic acid — 2.2 times ($p < 0,05$) compared with the control pathology group. The more pronounced effect of this therapy compared to other therapeutic schemes is characterized by the fact that edaravon has numerous pharmacological effects, in particular: anti-inflammatory, protective, and antioxidant effects, which prevents the death of retinal photoreceptors and affects the development of RRD.

The most pronounced therapeutic efficacy was found in rats treated with combination therapy with dexamethasone, resveratrol, erythropoietin, and edaravon for 7 days. In particular, the level of alanine decreased to a similar level in the group of intact animals. The level of aspartate decreased 3.7 times ($p < 0.05$) compared with the group of animals with RRD, but this figure was significantly different from the level of aspartate in intact rats. Changes in glutamic acid levels were also noted: it was significantly reduced 2.5 times ($p < 0.05$) relative to the control pathology group and significantly different from similar results of conditionally intact rats 3.14 times ($p < 0.05$).

Therefore, we can assume that the pronounced effect of combination therapy on the level of amino acids (aspartate, glutamate, alanine, and glycine) in the treatment of RRD is due to the pharmacological activity of the components of this therapeutic regimen and characterized by synergistic effects of each component in the key links of disease pathogenesis [17].

CONCLUSIONS

1. In animals modeling of RRD was observed a significant increase in the level of alanine, aspartate, glycine, glutamic acid compared with rats of the conditionally intact group. The obtained data can be explained by significant neurochemical changes of the glutamatergic system of the neuronal retina, which cause excitotoxicity (as a result of the massive release of neuronal glutamate) and structural changes.
2. In the study of the level of valine, histidine, tyrosine, phenylalanine, and methionine, it was found that these amino acids are not involved in the pathogenesis of RRD and their level does not change.
3. The most pronounced therapeutic efficacy was found in rats treated with combination therapy with dexamethasone, resveratrol, erythropoietin, and edaravon for 7 days. The pronounced effect of combination therapy on the level of amino acids (aspartate, glutamate, alanine, and glycine) in the treatment of RRD is due to the pharmacological activity of the components of this therapeutic regimen and characterized by synergistic effects of each component in the key links of disease pathogenesis.

Conflict of interest

All authors declare no conflict of interest.

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