Levytska H. V., Levytskyi I. M., Savytskyi I. V., Zaiats L. M., Sarakhan L. V. Investigation of amino acids' levels in the vitreous body of experimental animals in regmatogenic retinal detachment. Journal of Education, Health and Sport. 2021;11(11):414-422. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2021.11.11.039
https://apcz.umk.pl/JEHS/article/view/JEHS.2021.11.11.039
https://zenodo.org/record/7032672

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.

Received: 10.10.2021. Revised: 22.10.2021. Accepted: 30.11.2021.

INVESTIGATION OF AMINO ACIDS' LEVELS IN THE VITREOUS BODY OF EXPERIMENTAL ANIMALS IN REGMATOGENIC RETINAL DETACHMENT

H. V. Levytska[1], I. M. Levytskyi[1], I. V. Savytskyi[1], L. M. Zaiats[2], L. V. Sarakhan[3]

[1]International European University, Kyiv, Ukraine
[2]Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine
[3]Odessa National Medical University, Odesa, Ukraine

Abstract

The article presents the results of studying the levels of amino acids in the vitreous body of rats with rhegmatogenous retinal detachment at different stages of modeling the pathology (on the 3rd, 5th and 7th day). In animals with modeling RRD, was observed a significant increase in the level of alanine, aspartate, glycine, glutamic acid compared with rats of conditionally intact group; maximum changes in indicators were observed on the 7th day of the study. The obtained data are explained by significant neurochemical changes of the glutamatergic system of the neural retina, which cause excitotoxicity (as a result of massive release of neuronal glutamate) and structural changes. 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, so their level does not change. The obtained experimental data deepen the existing pathophysiological data on the pathogenetic links of rhegmatogenous retinal detachment in the early stages of its progression, which is important for practical ophthalmology to develop effective pharmacotherapy of this disease.

Key words: rhegmatogenous retinal detachment; amino acids; vitreous body.

ДОСЛІДЖЕННЯ РІВНІВ АМІНОКИСЛОТ В СКЛОВИДНОМУ ТІЛІ ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН ПРИ РЕГМАТОГЕННОМУ ВІДШАРУВАННІ СІТКІВКИ

Г. В. Левицька[1], І. М. Левицький[1], І. В. Савицький[1], Л. М. Заяць[2], Л. В. Сарахан[3]

[1]Міжнародний Європейський університет, Київ, Україна
[2]Івано-Франківський національний медичний університет, І-Франківськ, Україна
[3]Одеський національний медичний університет, Одеса, Україна

В статі представлені результати вивчення рівнів амінокислот в скловидному тілі щурів при регматогенному відшаруванні сітківки на різних термінах моделювання патолгії (на 3-у, 5-у та 7-у добу). Встановлено, що у тварин, яким моделювали РВС, спостерігали достовірне підвищення рівня аланіну, аспартату, гліцину, глутамінової кислоти порівняно із щурами умовно інтактної групи; максимальні зміни показників відмічено на 7-у добу дослідження. Одержані дані пояснюються значними нейрохімічними змінами глутаматергічної системи нейрональної сітківки, що викликають ексайтотоксичність (як результат масивного викиду нейронального глутамату) та структурні зміни. При дослідженні рівня валіну, гістидину, тирозину, фенілаланіну та метіоніну встановлено, що дані амінокислоти не беруть участь в патогенезі РВС, тому їх рівень не змінюється. Отже, можна припустити, що тривалість експериментального відтворення РВС має безпосередньо прямий негативний вплив на рівень амінокислот – тривала дія підвищеного рівня глутамату, аланіну та гліцину призводить до більш суттєвого пошкодження сітківки та незворотних змін в ній із розвитком некрозу. Одержані експериментальні дані поглиблюють існуючі патофізіологічні дані щодо патогенетичних ланок регматогенного відшарування сітківки на ранніх етапах їх прогресування, що має важливе значення для практичної офтальмології з метою розробки ефективної фармакотерапії даного захворювання.

Ключові слова: регматогенне відшарування сітківки; амінокислоти; скловидне тіло.

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 due to the operation of a complex photoreceptor apparatus [1].

Rhegmatogenous retinal detachment (RRD) occurs on the background of retinal rupture, as a result of which fluid from the VB enters to the retina. 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. Despite the rapid development and achievements of modern ophthalmic surgery, the problem of restoring vision in retinal detachment has been, is and will be one of the main causes of blindness and disability, as the prevalence of this disease ranges from 8.9 to 24.4 cases per 100 thousand. population per year, up to 30 % of cases - with bilateral lesions [1, 4].

Recently, the study of retinal neurochemical activity (in particular, determination of aminoacid levels) in normal and 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 damage [7].

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 massive release of methane changes [6, 7, 10].

According to the literature data were confirmed experimentally in a clinical study studying the aminoacid profile of the vitreous and vitreal content in patients with RRD with different clinical characteristics [4].

A more in-depth study of amino acid levels in VB rats in experimental modeling of RRD can supplement existing data on the pathogenesis of this disease and contribute to the correct choice of appropriate pathogenetic therapy.

The aim of the study was to establish the changes in amino acid levels in the VB rats under the conditions of experimental RRD.

Materials and methods. Experimental studies were performed on 36 brown Norwegian male rats, which were divided into 2 groups (18 animals in each group): 1 group – conditionally intact control (without retinal detachment) – animals that were underwent paracentesis of the anterior chamber with 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 [12] (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.

The study of the amino acid composition of VB changes in rats with simulated RRD was performed on the 3rd, 5th and 7th day of the experiment, which allows a more thorough study of pathogenetic changes in the studied pathology. For this aim, the animals were removed from the experiment sequentially – 6 animals in each of these terms, both from the group of control pathology and from the group of conditionally intact control.

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 2.6x250. Detection of amino acids was performed at 570 nm, except for proline (which was determined at 440 nm). The results were calculated by the formula using standard aminogram data, which was used to determine the content of a specific amino acid and expressed in ng/ml [2].

During the work with animals we took into account 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 the protection of animals used for scientific purposes, the Law of Ukraine "On protection of animals from cruel treatment" № 440-IX of 14.01.2020 [9].

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 [3].

Results and discussion. The study of amino acid levels in the VB of rats was carried out in the dynamics: on the 3rd, 5th and 7th day of experimental modeling.

It was found that already on the 3rd day of experimental modeling of RRD in rats there were slight changes in the concentration of valine, histidine, tyrosine, phenylalanine and

methionine, but their level did not differ from the group of conditionally intact animals (Table 1). On the 5th day of the study, the levels of the amino acids also increased significantly.

Table 1

The levels of valine, histidine, tyrosine, phenylalanine, methionine in vitreous body of rats

($X \pm S_X$, n=6)

Rats' groups	valine, ng/ml	histidine, ng/ml	tyrosine, ng/ml	phenylalanine, ng/ml	methionine, ng/ml
on the 3rd day of the experiment					
Conditionally intact animals	1,5±0,4	1,1±0,2	0,081±0,042	0,021±0,012	0,10±0,1
Control pathology	1,8 ±0,3	1,2±0,26	0,082±0,047	0,029±0,012	0,10±0,1
on the 5th day of the experiment					
Conditionally intact animals	1,6±0,6	1,26±0,24	0,082±0,041	0,020±0,015	0,11±0,1
Control pathology	2,1±0,29	1,32±0,31	0,084±0,042	0,030±0,016	0,12±0,11

Note. n – the number of animals in each group (n=6).

The level of alanine on the 3rd day of the study increased in 1.5 times ($p<0.05$), on the 5th day – in 1.4 times ($p<0.05$); the level of aspartate on the 3rd day of the study increased in 9.0 times ($p<0.05$), on the 5th day – in 10.7 times ($p<0.05$); the level of glycine on the 3rd day increased in 2.1 times ($p<0.05$), on the 5th day – in 2.2 times ($p<0.05$); the level of glutamic acid on the 3rd day increased in 7.0 times ($p<0.05$), on the 5th day – in 8.0 times ($p<0.05$) compared with similar indicators of conditionally intact rats. In the study of arginine levels, no significant differences were found (Table 2).

Table 2

The levels of alanine, arginine, aspartate, glycine and glutamic acid in vitreous body of rats

($X \pm S_X$, n=6)

Rats' group	alanine, ng/ml	arginine, ng/ml	aspartate, ng/ml	glycine, ng/ml	glutamic acid, ng/ml
on the 3rd day of the experiment					
Conditionally intact animals	2,7±0,21	0,5±0,1	2,0±0,42	3,8±0,6	1,34±0,27
Control pathology	4,0±0,27*	0,75±0,12	18,3±1,8*	7,8±1,1*	9,4±1,8*
on the 5th day of the experiment					
Conditionally intact animals	3,0±0,25	0,7±0,1	2,1±0,44	4,2±0,7	1,37±0,28
Control pathology	4,3±0,29*	0,8±0,11	22,4±2,1*	9,2±1,5*	11,0±1,6*

Notes:

1. * - $p<0,05$ compared with the intact group of animals.

2. n – the number of animals in each group (n=6).

On the 7th day of the experiment in the conditionally intact control group it was found

that the concentration of amino acids in VB was: 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 (Fig. 1, Fig. 2).

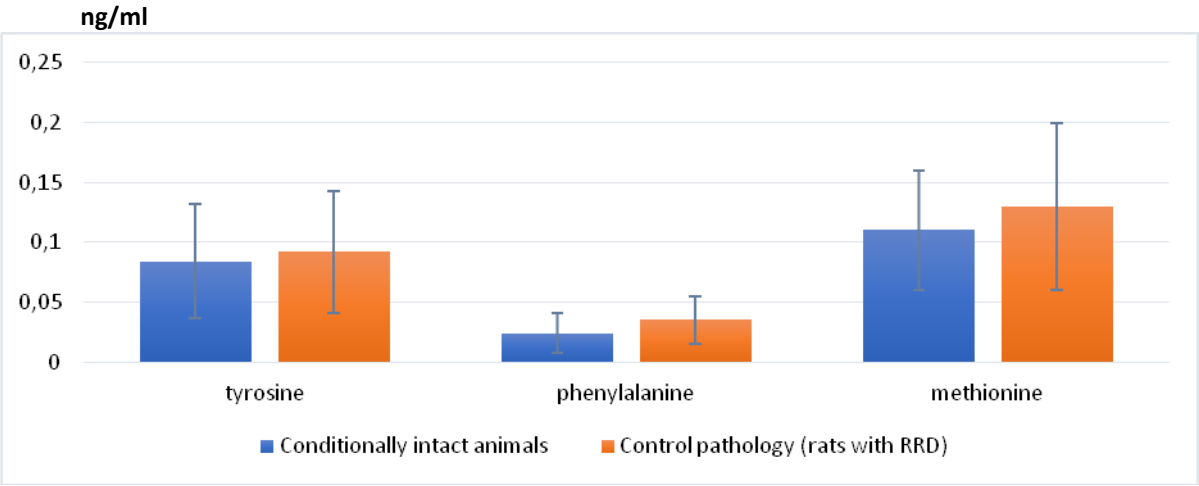

Fig. 1. The level of some amino acids (tyrosine, phenylalanine, methionine) in the vitreous body of a conditionally intact group and in rats with a simulated RRD on the 7th day of the experimental study.

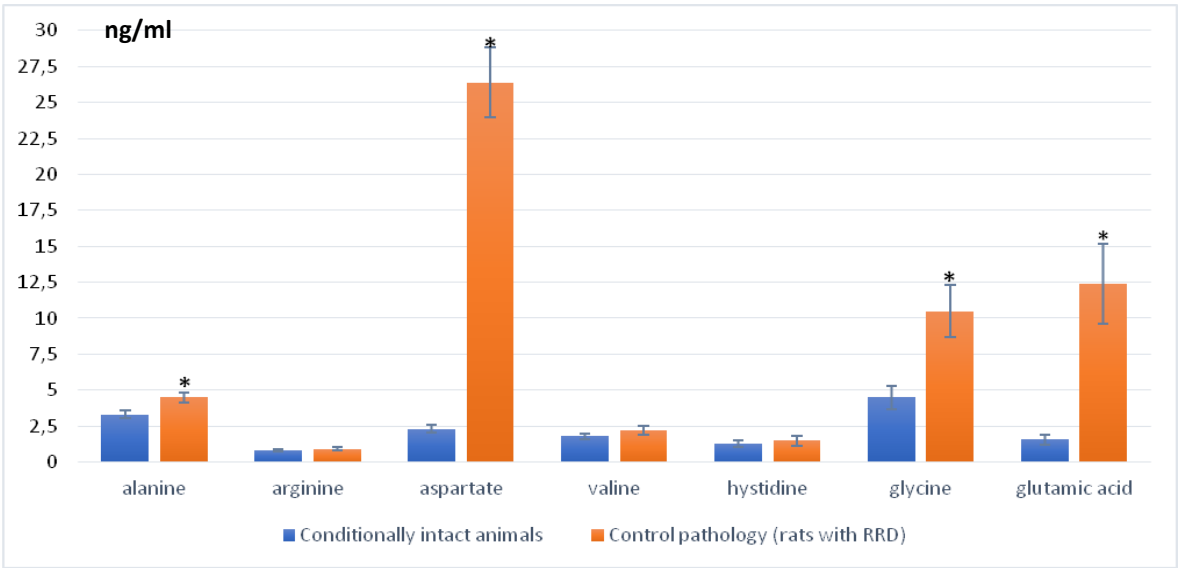

Fig. 2. The level of amino acids in the vitreous body of the conditionally intact group and in rats with simulated RRD on the 7th day of the experimental study

Notes:

1. * - $p<0,05$ compared with the intact group of animals.

2. n – the number of animals in each group (n=6).

Elevations in tyrosine (0.092±0.051), phenylalanine (0.035±0.02) and methionine (0.13±0.07) were observed in the group of animals simulated by RRD on the 7th day of the study. However, their level did not differ from similar data in the group of conditionally intact rats, which indicates that these amino acids are not involved in the pathogenesis of RRD, so their level does not change (Fig. 1).

In animals that simulated RRD on the 7th day of the study showed a significant increase in certain amino acids: the level of alanine increased in 1.4 times, aspartate – in 11.5 times ($p<0.05$), glycine – in 2.3 times ($p<0.05$), glutamic acid – in 7.9 times ($p<0.05$) compared with rats of conditionally intact group. Levels of other amino acids increased insignificantly (Fig. 2).

The obtained data are explained by the fact that as a result of retinal detachment there are significant neurochemical changes: increased levels of glutamate, glycine, alanine, aspartate due to changes in the glutamatergic system of the retina, which causes massive release of neuronal glutamate and causes concomitant changes in its metabolism. In turn, the release of neuronal glutamate causes excitotoxicity and initiates structural changes [6].

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 eventually lead to apoptosis or necrosis [7].

Accumulation of glutamate and aspartate in the VB of RVS rats may be the result of their release from dead retinal ganglion cells, leading to further neuronal damage. Increased excitotoxicity of glutamate and aspartate in VB is associated with ischemic processes in the optic nerve [10]. Elevated glycine levels may be due to retinal ischemia caused by RRD in rats [11]. Our results in modeling RRD in rats correlate with data obtained by other researchers [6, 7, 10, 11].

Thus, we can assume that the duration of experimental reproduction of RRD has a direct impact on amino acid levels – prolonged exposure to elevated levels of glutamate, alanine and glycine leads to more significant retinal damage and irreversible changes in it with necrosis [11, 13, 14].

The obtained data provide an opportunity to deepen the existing knowledge about the pathogenesis of RRD in order to develop and implement more effective pharmacocorrection of this disease.

Conclusions:

1. In animals with modeling RRD, was observed a significant increase in the level of alanine, aspartate, glycine, glutamic acid compared with rats of conditionally intact group;

maximum changes in indicators were observed on the 7th day of the study. The obtained data are explained by significant neurochemical changes of the glutamatergic system of the neural retina, which cause excitotoxicity (as a result of 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, so their level does not change.

3. The obtained experimental data deepen the existing pathophysiological data on the pathogenetic links of rhegmatogenous retinal detachment in the early stages of its progression, which is important for practical ophthalmology to develop effective pharmacotherapy of this disease.

References:

1. Avanesova T. A. Rhegmatogenous retinal detachment: the current state of the problem. *Oftalmologiya.* 2015. Vol. 1 (12). P. 24–32. (In Russian)
2. Baratova L. A. The principle of operation of the amino acid analyzer and determination of the amino acid composition of the protein. M.: 1988, 36 p. (In Russian)
3. Methods of statistical processing of medical data: method. reccom. / A. G. Kochetov, O. V. Liang, I. V. Zhirov et al. M.: RKNPK, 2012. 42 p. (In Russian)
4. Pasechnikova N. V., Levitskaya G. V. Amino acid profile of vitreous and vitreal contents in patients with rhegmatogenous detachment with different clinical characteristics. *Oftalmologiya.* 2014. Vol. 2 (15). P. 45–55. (In Russian)
5. Reznikov O. G., Solovyov A. I., Stefanov O. V. Biotic examination of preclinical and other scientific studies performed on animals: method. recommendations. *Bulletin of Pharmacology and Pharmacy.* 2006. Vol. 7. P. 47–61. (In Ukrainian).
6. Ambati J. Elevated gamma-aminobutyric acid, glutamate, and vascular endothelial growth factor levels in the vitreous of patients with proliferative diabetic retinopathy. *Arch. Ophthalmol.* 1997. Vol. 115. P. 1161–1166.
7. Amino-acid levels in subretinal and vitreous fuid of patients with retinal detachment / K. M. Bertram, D. V. Bula, J. S. Pulido et al. *Eye*. 2008. Vol. 22. P. 582–589.
8. Drug Delivery to the Posterior Segment of the Eye: Biopharmaceutic and Pharmacokinetic Considerations / R. Varela-Fernandez, V. Diaz-Tome, A. Luaces-Rodriguez et al. *Pharmaceutics.* 2020. Vol. 12 (269). doi:10.3390/pharmaceutics 12030269.
9. https://zakon.rada.gov.ua/laws/show/3447-15
10. Increased glutamate levels in the vitreous of patients with retinal detachment /

R. M. Diederen, E. C. La Heij, N. E. Deutz et al. *Exp. Eye Res*. 2006. Vol. 83 (1). P. 45–50.

11. Memantine is neuroprotective in a rat model of pressure-induced retinal ischemia / W. A. Lagrèze, R. Knörle, M. Bach et al. *Invest. Ophthalmol. Vis. Sci.* 1998. Vol. 39. P. 1063–1066.

12. Relocalization of apoptosis-inducing factor in photoreceptor apoptosis induced by retinal detachment in vivo / T Hisatomi et al. *The American Journal of Pathology*. 2001. № 4 (158). P. 1271–1278.

13. Simvastatin, edaravone and dexamethasone protect against kainite-induced brain endothelial cell damage / L. Barna, F. R. Walter, M. A. Deli et al. *Fluids and Barriers of the CNS*. 2020. Vol. 5 (17). doi: https://doi.org/10.1186/s12987-019-0166-1

14. Vitreous antioxidants, degeneration, and vitreo-rethinopathy: exploring the links / E. Ankamah, J. Sebag, E. Ng, J. M. Nolan. *Antioxidants (Basel)*. 2020. Vol. 9 (1). doi: 10.3390/antiox9010007