Levytskyi I. M., Savytskyi I. V., Levytska G. V., Miastkivska I. V. Changes in the functional state of the endothelium in conditions of experimental rhegmatogenous retinal detachment and its correction. Journal of Education, Health and Sport. 2017;7(11):409-418. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.2784034
http://ojs.ukw.edu.pl/index.php/johs/article/view/6911

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26.01.2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 06.11.2017. Revised: 20.11.2017. Accepted: 30.11.2017.

CHANGES IN THE FUNCTIONAL STATE OF THE ENDOTHELIUM IN CONDITIONS OF EXPERIMENTAL RHEGMATOGENOUS RETINAL DETACHMENT AND ITS CORRECTION

I. M. Levytskyi, I. V. Savytskyi, G. V. Levytska, I. V. Miastkivska

Odessa National Medical University

Abstract

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

Purpose: investigation of endothelial dysfunction in rhegmatogenous retinal detachment pathogenesis and analysis of correction methods effectiveness.

Von Willebrand factor (VWF) is common marker of endothelial dysfunction. It was established increase in its level in the 7th day of the research in all groups in which rats were modeled rhegmatogenous retinal detachment. Differences in the comparison of these groups among themselves have not been identified.

At 14th day, it is observed endothelial dysfunction development in group 2 (differences in comparison with the data of intact animals already at the level of significance $p < 0.01$). In group of rats receiving a two-component correction, decrease in the level of VWF was found. In the group receiving the three-component correction, the level of the von Willebrand factor is more strongly approximated to the norm ($p < 0.05$ when comparing the data of this group and the animals that did not undergo correction of the experimental RRD)

On 21st day no differences were found when comparing the results of intact animals with the data of each group receiving correction of the modeled RRD, indicating the

normalization of endothelial cells functioning. It is worth noting that in the group in which animals received cytokoline, D-asparagine and L-arginine (group №4), most expressed normalized functional state of the endothelium, as evidenced by the differences in comparison with the data of 2nd and 3rd groups on levels of significance $p < 0.01$

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

Introduction

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 [1].

Incidence of RRD increases with age and reaches 0.06% of the population over the age of 60 [2, 3]. Frequency of RRD development in untreated retinal gaps is 48-55% and when laser-blocked retinal gaps are reduced to 1.84-7.8% [4].

However, according to Folk J.C. et al [5] prophylactic surgical treatment correction does not reduce the likelihood of the rhegmatogenous retinal detachment development.

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. Taking into account the above, it is necessary to analyze the vessel endothelium dysfunction for better understanding of the pathogenetic parts of the retinal detachment, as well as for the competent selection of RRD correction methods.

Purpose: investigation of endothelial dysfunction 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.

Level of von Willebrand factor was determined by enzyme-linked immunosorbent assay.

Research results

Von Willebrand factor (VWF) is common marker of endothelial dysfunction. It was established increase in its level in the 7th day of the research in all groups in which rats were modeled rhegmatogenous retinal detachment (Table 1). Differences in the comparison of these groups among themselves have not been identified.

Table 1. Dynamics of von Willebrand factor level in rats blood at experimental rhegmatogenous retinal detachment and its correction at the 7th day of the research.

	Intact animals 1	7th day		
		Group №2 2	Group №3 3	Group №4 4
VWF	84.2±1.1	89.2±1.2 p_{21}*	89.1±1.0 p_{31}*	89.1±1.3 p_{41}*

Footnote: * – p<0,05

At 14th day, it is observed endothelial dysfunction development in group 2 (differences in comparison with the data of intact animals already at the level of significance p <0.01). In group of rats receiving a two-component correction, decrease in the level of VWF was found. In the group receiving the three-component correction, the level of the von Willebrand factor is more strongly approximated to the norm (p <0.05 when comparing the data of this group and the animals that did not undergo correction of the experimental RRD) (Table 2).

Table 2. Dynamics of von Willebrand factor level in rats blood at experimental rhegmatogenous retinal detachment and its correction at the 14th day of the research.

	Intact animals 1	14th day		
		Group №2 5	Group №3 6	Group №4 7
VWF	84.2±1.1	90.9±0.9 p_{51}**	87.21±1.4 p_{61}*	86.0±1.2 p_{71}* p_{75}*

Footnote: * – p<0,05

On 21st day no differences were found when comparing the results of intact animals with the data of each group receiving correction of the modeled RRD, indicating the

normalization of endothelial cells functioning. It is worth noting that in the group in which animals received cytokoline, D-asparagine and L-arginine (group №4), most expressed normalized functional state of the endothelium, as evidenced by the differences in comparison with the data of 2nd and 3rd groups on levels of significance p <0.01 (Table 3).

Table 3. Dynamics of von Willebrand factor level in rats blood at experimental rhegmatogenous retinal detachment and its correction at the 21st day of the research.

	Intact animals 1	21st day		
		Group №2 8	Group №3 9	Group №4 10
VWF	84.2±1.1	91.2±1.2 p_{81}**	86.3±0.7 p_{98}*	85.4±0.5 $p_{10\text{-}8}$**

Footnote: * – p<0,05

On fig. 1 is given full description of changes in the level of endothelial dysfunction marker during the whole experiment. It is clearly confirmed by its progression in animals that did not receive correction against the background of the simulated pathology. Most pronounced normalization of endothelial cells functioning was found in the group, which received a nitric oxide donor as a part of the complex correction (Group № 4)

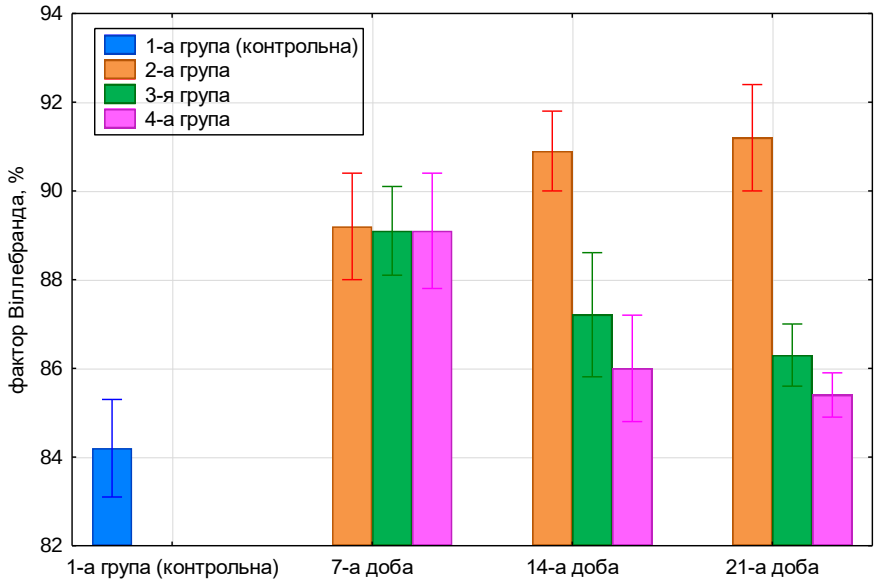

Figure 1. Change von Willebrand factor level in the blood of experimental animals in the dynamics after the experimental rhegmatogenous retinal detachment and its correction

Research result discussion

It is known that under inflammatory mediators influence, endothelial cells lose their anti-adhesive and anti-inflammatory properties, forming a prothrombogenic surface that stimulates inflammation development [6]. According to the literature, a correlation between the increase of the von Willebrand factor concentration in the blood and the degree of endothelial dysfunction, as in the experiment [7], and in a number of clinical observations [8] has been proved. In analyzing the factor Willebrand level in our research, it is confirmed that the rhegmatogenous retinal detachment leads to endothelial dysfunction development: increase in values of investigated indicator in the background of the simulated pathology has been revealed. So in the second group on the 7th day the level of VWF increased by 5.9%, by the 14th - by 8%, and by the 21st day - by 8.3%.

On the background of correction in both groups, a decrease in von Villebrand factor level was found. In the third group at day 14, its activity improved by 4.1%, and by the 21st at 5.4% compared with the data of group №2. And in the fourth group, in comparison with the second, its level decreased by 5.4% on the 14th day and by 6.4% on the 21st. Noteworthy fact that in group receiving cytokoline, D-asparagine and L-arginine results are better than in the group receiving only cytokoline and D-asparagine. Foregoing proves the expediency and effectiveness of L-arginine inclusion in the composition of corrective agents in the rhegmatogenous retinal detachment.

Let's take closer look at the L-arginine properties and the mechanisms of its influence on functional state of the endothelial cells.

Violation of nitric oxide synthesis can lead to endothelial dysfunction (ED), which in turn plays important role as regulator of biological effects [9]. In vascular homeostasis, NO is responsible for vascular tone, apoptosis and regulation, correction of oxidative processes and angioprotective properties. Nitric oxide is also a classic peripheral vasodilator [10]. It is generally known that arginine is the main substrate for NO synthesis [11]. This amino acid is very important for many metabolism reactions, but its main role is to synthesize nitrogen oxide [12]. Therefore, it is required L-arginine for prevention and correction of endothelial dysfunction and improvement of blood supply, [13, 14]. Its protector actions are explained as follows: it is a precursor of gamma-Aminobutyric acid, L-citrulline, L-ornithine, spermitin, L-glutathione and other compounds [15, 16]. For the first time, this amino acid was isolated by E. Schulze and E. Steiger in 1886, and the structure was finally identified by E. Schulze and E. Winterstein in 1897. It consists of a positively charged Rh group, which is also an integral part of the body's main proteins [17]. L-arginine in the body is converted into two

alternative pathways, which can take place simultaneously: with the participation of NO-synthase - oxidative, with the subsequent formation of L-citrulline and NO; and with the participation of arginase I - non-oxide, with subsequent formation of L-ornithine and urea [13, 14, 18].

L-arginine takes active part in the synthesis of nitric oxide, polyamines and a number of anabolic hormones [16]. This amino acid is absorbed by the body in the small intestine and then transported to liver, which is mostly utilized in the ornithine cycle, and the other part becomes a substrate for the synthesis of NO [19]. In a small amount of L-arginine, which is in the plasma, selectively improves the vessels endothelial function, at medium concentrations, causes pronounced vasodilatation due to stimulation of insulin secretion and growth hormone, while high levels of L-arginine contribute to nonspecific vasodilatation [10, 13, 19, 20].

L-arginine activates nitric oxide synthesis and reduces endothelial dysfunction manifestations by increasing endothelial nitric oxide synthase (eNO-S) activity [21]. It also prevents tetrahydrobiopterin oxidation - major co-factor NO-S. By inhibiting the oxidation of low density lipoproteins (LDL), which lowers NO, indicated amino acid breaks the eNO-S complex with caveolins (potentiated by oxidized LDL), which is an inhibitor of NO-S activity [14, 20, 22].

L-arginine activates NO action by direct antioxidant activity, increase its bio activity, while reducing norepinephrine activity and stimulating histamine release from the main cells, thereby enhancing the vasodilator effect [23]. In vivo, L-arginine reduces the level of NO-S-mediated superoxide [23]. It also helps to unlock eNO-S expression, which occurs under the action of asymmetric dimethylargrinine and L-NG-monomethyl arginine, and also reduces the concentration of endothelin-1, an important modulator of endothelial dysfunction and vasoconstrictor [13, 14, 24].

L-arginine-NO system, directly affects state of the endothelium, thus provides the functioning of the cardiovascular system [25], optimizes the function of the endothelium against hypercholesterolemia [26], which is important in apoptosis, balancing inflammatory response, and protection against oxidative damage [27]. L-arginine affects microcirculation and systemic circulation during infusion against background of experimental hemorrhagic shock, improves myocardial function, and thus contributes to the survival of animals [28]. Positive therapeutic effect of L-arginine correction of the endothelium in patients with obliteration atherosclerosis is characterized by prolonged action [13, 14, 29].

Conclusions :

1. It was found that von Willebrand factor is informative indicator of endothelial dysfunction in experimental rhegmatogenous retinal detachment (established differences at the significance level $p < 0.05$ for the 7th day and $p < 0.01$ for the 14th and 21st day of the experiment).

2. It has been found that endothelial dysfunction is important pathogenetic link of rhegmatogenous retinal detachment.

3. Three-component correction of the experimental rhegmatogenous retinal detachment, which consisted of cytokoline, D-asparagine and L-arginine, demonstrated its effectiveness in normalizing the von Willebrand factor level.

4. It has been established that use of L-arginine, which is a nitric oxide donor, is effective in endothelial dysfunction correction against the background of experimental rhegmatogenous retinal detachment.

References

1. Libman ES, Shahova EV. Sostoyanie i dinamika slepoty i invalidnosti vsledstvie patologii organa zreniya v Rossii. Tezisy dokladov VII Sezda oftalmologov Rossii. 2000:209-214.

2. Anina AV, Martoplyas KV. Patologiya setchatoj obolochki i zritelnogo nerva sredi vzroslogo naseleniya Ukrainy. Tezi dopovidej naukovo-praktichnoyi konferenciyi z mizhnarodnoyu uchastyu “Nove v oftalmologiyi”, prisvyachena 130 – richchyu z dnya narodzhennya akademika V.P.Filatova. Odesa. 2005:151 –152.

3. Piri A, Van Gejnigen A. Biohimiya glaza. Medicina. 1968:399

4. Foos RY. Vitreous base, retinal tufts, and retinal tears: Pathogenetic relationships. In R.C. Pruett, C.D.J. Regan: Retina Congress. 1974:259.

5. Folk JC, Blodi CF. Treatment of macular hole retinal detachments with intravitreal gas. Am J Ophthalmol. 1984;15:811–814.

6. Voznyuk LA, Pivtorak KV, Semenenko SI. Faktor Villebranda yak kriterij zapalennya i disfunkciyi endoteliyu u hvorih na ishemichnu hvorobu sercya. Visnik problem biologiyi i medicini. 2012;1;2(92):39-42.

7. Malaya LT, Korzh AN, Balkovaya LB. Zndotelialnaya disfunkciya pri patologii serdechno-sosudistoj sistemi. - H. : Torsing, 2000. - 432 s.

8. Polivoda SN, Cherepok AA. Faktor Villebranda kak marker endotelialnoj disfunkcii u pacientov s zabolevaniyami serdechno-sosudistoj sistemy. Ukrayinskij revmatologichnij zhurnal. 2000;1:13-18.

9. Chatterjee A, Catravas JD. Endothelialnitricoxide (NO) and its pathophysiologic regulation. Vascul. Pharmacol. 2008;49(4-6):134-140.

10. Gudyrev OC, Radzhkumar DSR , Fajtelson AB i dr. Osteoprotektivnoe dejstvie L-arginina pri eksperimentalnom osteoporoze. Nauchnye vedomosti BelGU. Ser. Medicina. Farmaciya. 2012;20(3)-22(141):94-97.

11. Reutov VP, Sorokina EG, Ohotin VE, Kosicyn NS. Ciklicheskie prevrasheniya oksida azota v organizme mlekopitayushih. Nauka. 1997;156.

12. Malaya L.T, Korzh AN , Balkovaya LB . Endotelialnaya disfunkciya pri patologii serdechno-sosudistoj sistemy. Forsing. 2000:245.

13. Serdyuk VM, Savickij IV, Semenko VV. Osoblivosti vmistu NOh v sirovatci krovi i v sechi ta aktivnist NO-sintazi v ochnomu yabluci za eksperimentalnoyi DR. Arhiv oftalmologiyi Ukrayini. 2017;5(3 (9)):75-80.

14. Savickij IV, Semenko VV, Serdyuk VM. Metabolichna korekciya eksperimentalnoyi DR. Oftalmologichnij zhurnal. 2017;6:72-77.

15. Marini JC. Arginine and ornithine are the main precursors for citrulline synthesis in Mice. J. Nutr. 2012;142(3):572–580.

16. Gajda GZ, Stasyuk NYe, Gonchar MV. Metodi analizu L-argininu. Viotechnologia acta. 2014;7(1):31-39.

17. Yokoro M, Suzuki M, Murota K, Otsuka C, Yamashita H, Takahashi Y, Tsuji H, Kimoto M. Asymmetric dimethylarginine, anendogenous NOS inhibitor, metabolized in raterythrocytes. Biosci. Biotechnol. Biochem. 2012;76(7):1334-1342.

18. Oka RK, Szuba A, Giacomini JC, Cooke JP. A pilot study of L-arginine supplementation on functional capacity in peripheralarterial disease. Vasc. Med. 2005;10(4):265-274.

19. Markov HM. Oksidantnyj stress i disfunkciya endoteliya. Patol.fiziologiya i eksperim. terapiya. 2005;4:5-9.

20. Loscalzo J. L-arginine and Atherothrombosis. J. Nutr. 2004;134:2798-2800.

21. Boger RH. The pharmacodynamics of L-arginine. J. Nutr. 2007;137:1650-1655.

22. Boger RH, Bode-Boger SM, Mugge A, Kienke S, Brandes R, et al. Supplementation of hypercholesterolaemicrabbits with L-arginine reduces the vascular release of superoxideanions and restores NO production. Atherosclerosis. 1995;117:273-84.

23. Huk I, Nanobashvili J, Neumayer C, Punz A, Mueller M, et al. L-arginine treatment alters the kinetics of nitricoxide and superoxide release and reduces ischemia, reperfusion injury in skeletal muscle. Circulation. 1997;96:667-75.

24. Zweier JL, Samouilov A, Kuppusamy P. Non-enzymatic nitricoxide synthesis in biological systems. Biochim. Biophys. Acta. 1999;1411:250-262.

25. Morikawa E, Moskowitz MA, Huang Z, et al. L-arginine in fusion promotes nitric oxide-depend entvasodilation, increases regional cerebral blood flow, and reduces in farction volume in the rat. Stroke. 1994;25:429-435

26. Marchesi S, Lupattelli G, Siepi D, et al. Oral L-arginine administration attenuates post prandialendothelial dysfunction in young healthy males. Clin. Pharm. Ther. 2001;26:343-349.

27. Lyons CR. The role of nitricoxidein in flammation. Adv. Immunol. 1995;60:323-371.

28. Lee C, Li D, Channon K, Paterson DJ. L-arginine supplementation reduces cardiacnoradrenergic neurotransmission in spontaneously hypertensive rats. J. Mol. CellCardiol. 2009;47(1):149-155.

29. Grishina GV, Gerbut KA. Vliyanie L-arginina na sistemu krovoobrasheniya pri infuzionnoj terapii eksperimentalnogo gemorragicheskogo shoka. Aktualnye voprosy transfuziologii i klinicheskoj mediciny. 2015;1:53-55.