УДК 616.894-053.8-021.6-092:612.822.56
https://doi.org/10.20538/1682-0363-2020-4-46-52

Aberrant angiogenesis in brain tissue in experimental Alzheimer's disease

Gorina Ya.V., Komleva Yu.K., Osipova E.D., Morgun A.V., Malinovskaya N.A., Lopatina O.L., Salmina A.B.

Krasnoyarsk State Medical University named after Prof. V.F. Voino-Yasenetsky
1, Partizana Zheleznyaka Str., Krasnoyarsk, 660022, Russian Federation

ABSTRACT

The aim was to study the molecular mechanisms of the violation of the structural and functional integrity of the blood-brain barrier in chronic neurodegeneration of the Alzheimer's type associated with the development of cerebral angiopathy.

Materials and methods. The transgenic model of Alzheimer's disease is the B6SLJ-Tg line mice (APPSwFlLon, PSEN1*M146L*L286V) 6799Vas group which includes 9 months aged males. The control group included C57BL/6xSJL mice, males aged 9 months.

Results. The total length of the vessels in the area of the dentate gyrus is 2.5 times greater in transgenic animal models of Alzheimer's disease than in animals of the control group ($p < 0.01$). The average diameter of blood vessels in all areas of the hippocampus is smaller compared with the control ($p < 0.05$). Transgenic modeling of neurodegeneration in the CA2 zone of the hippocampus increases the relative area of tissue with increased permeability of blood-brain barrier (BBB) (17.80 [9.15; 36.75]) compared to control (1.38 [0.04; 7.60]) at $p < 0.05$. A similar difference ($p < 0.05$) is also observed in the hippocampal area CA1. A tendency ($p > 0.05$) to decrease the number of CD31+ endothelial cells in the dentate gyrus of the hippocampus (21.52 [17.56; 24.50]) in animals of the experimental group compared with the control group (23.08[21.18; 29.84]) was detected. A similar situation is observed in the CA2 and CA3 areas of the hippocampus.

Conclusion. Neurodegenerative changes in the hippocampus of animals with a transgenic AD model are associated with impaired microcirculation in the brain tissue as a result of a reduction in the diameter and branching of blood vessels, and damage and increased permeability of BBB.

Key words: angiogenesis, blood-brain barrier, CD31, Alzheimer's disease.

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of funding. This work was supported by the grant awarded by the President of the Russian Federation for state support of leading scientific schools of the Russian Federation (6240.2018.7).

Compliance with ethical standards. The experiments were carried out after consideration of the application and protocol for the use of laboratory animals at a meeting of the bioethical commission at the local Ethics Commission at Krasnoyarsk State Medical University of the Russian Ministry of Health (Protocol No. 3 of October 08.10.2018).

For citation: Gorina Ya.V., Komleva Yu.K., Osipova E.D., Morgun A.V., Malinovskaya N.A., Lopatina O.L., Salmina A.B. Aberrant angiogenesis in brain tissue in experimental Alzheimer's disease. *Bulletin of Siberian Medicine.* 2020; 19 (4): 46–52. https://doi.org/10.20538/1682-0363-2020-4-46-52.

✉ *Gorina Yana V.,* e-mail: yana_20@bk.ru.

Аберрантный ангиогенез в ткани головного мозга при экспериментальной болезни Альцгеймера

Горина Я.В., Осипова Е.Д., Моргун А.В., Малиновская Н.А., Комлева Ю.К., Лопатина О.Л., Салмина А.Б.

Красноярский государственный медицинский университет (КрасГМУ) имени профессора В.Ф. Войно-Ясенецкого
Россия, 660022, г. Красноярск, ул. Партизана Железняка, 1

РЕЗЮМЕ

Цель – изучение молекулярных механизмов нарушения структурно-функциональной целостности гематоэнцефалического барьера (ГЭБ) при хронической нейродегенерации альцгеймеровского типа, ассоциированной с развитием церебральной ангипопатии.

Материалы и методы. Опытная группа – генетическая модель болезни Альцгеймера (БА) – мыши линии B6SLJ -Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas, самцы в возрасте 9 мес. Контрольная группа – мыши линии C57BL/6 x SJL, самцы в возрасте 9 мес.

Результаты. У животных с генетической моделью БА в зубчатой извилине гиппокампа общая длина сосудов в 2,5 раза больше, чем у контрольной группы ($p < 0,01$), при этом средний диаметр сосудов во всех областях гиппокампа меньше по сравнению с контролем ($p < 0,05$). Выявлено, что при генетическом моделировании нейродегенерации в CA2 зоне гиппокампа наблюдается увеличение относительной площади ткани с повышенной проницаемостью ГЭБ (17,80 [9,15;36,75]) по сравнению с контролем (1,38 [0,04;7,60]) при $p < 0,05$. Подобное различие ($p < 0,05$) наблюдается и в зоне CA1 гиппокампа. У животных опытной группы выявлена тенденция ($p > 0,05$) к снижению количества CD31+ эндотелиальных клеток в зубчатой извилине гиппокампа (21,52 [17,56; 24,50]) по сравнению с контролем (23,08 [21,18; 29,84]). Аналогичная ситуация наблюдается в зонах CA2 и CA3 гиппокампа.

Заключение. Нейродегенеративные изменения в гиппокампе животных с генетической моделью БА ассоциированы с нарушением микроциркуляции в ткани головного мозга в результате сокращения диаметра и разветвленности сосудов, повреждения и повышения проницаемости ГЭБ.

Ключевые слова: ангиогенез, гематоэнцефалический барьер, CD31, болезнь Альцгеймера.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Работа выполнена при поддержке гранта Президента РФ для государственной поддержки ведущих научных школ РФ (RSh-6240.2018.7).

Соответствие принципам этики. Исследование одобрено локальным этическим комитетом КрасГМУ (протокол № 3 от 08.10.2018).

Для цитирования: Горина Я.В., Осипова Е.Д., Моргун А.В., Малиновская Н.А., Комлева Ю.К., Лопатина О.Л., Салмина А.Б. Аберрантный ангиогенез в ткани головного мозга при экспериментальной болезни Альцгеймера. *Бюллетень сибирской медицины*. 2020; 19 (4): 46–52. https://doi.org/10.20538/1682-0363-2020-4-46-52.

INTRODUCTION

Alzheimer's disease (AD) is a common neurodegenerative disease among older people, characterized by the accumulation of beta-amyloid plaques, neurofibrillary tangles, and death of neuronal cells [1]. Experimental data established using magnetic resonance imaging indicate that AD without concomitant pathological disorders is much less common than dementia, accompanied by pronounced vascular changes [2]. This confirms the significant contribution of vascular disorders to the development of cognitive dysfunction and, as a consequence, neurodegeneration.

It is believed that beta-amyloid (Aβ) contributes to the damage to microvessels, the development of cerebral amyloid angiopathy (CAA), rupture of the vascular wall, and impaired cerebral perfusion [3]. Thus,

as a result of a study of the brain of patients with AD with multiple cerebral microbleeds, low levels of Aβ in the cerebrospinal fluid were revealed. This is probably the result of an increased cerebral intravascular deposition of Aβ, which leads to a violation of the integrity of the vascular wall, causing microbleeding [4]. In addition, studies on two models of transgenic lines of mice with stroke (APPswe / PS1dE9 and tg2576) showed that cerebrovascular disorders affect the clearance of Aβ and, therefore, contribute to its deposition in the brain area with pronounced damaged blood vessels [5]. Also, brain damage caused by impaired cerebral circulation increases the expression of amyloid beta precursor protein (APP) and, therefore, Aβ cleavage. Thus, against the background of Aβ deposition, which causes cerebrovascular dysfunction, subsequent ischemia can enhance the expression of APP and Aβ cleavage, forming positive feedback and leading to a violation of the structural and functional integrity of the neurovascular unit (an integrated unit that consists of microvascular endothelial cells functionally associated with neurons, astrocytes, pericytes, and extracellular matrix components) [6].

It is important to note that with age, the permeability of the blood-brain barrier increases in patients with vascular dementia, namely, in sections of brain tissue (hippocampus and cortex), and an accumulation of neurotoxic blood proteins (thrombin, albumin, and immunoglobulins) is observed [7]. In addition, a violation of the BBB integrity in the hippocampus, especially the CA1 area and the dentate gyrus, correlates with the development of cognitive dysfunction and the destruction of pericytes [8].

Thus, a violation of the BBB integrity may contribute to the progression of AD associated with the development of CAA. However, the molecular mechanisms underlying the pathogenesis are not completely clear. The purpose of this study is to study the mechanisms of violation of the structural and functional integrity of the BBB in chronic Alzheimer's type neurodegeneration associated with the development of CAA.

MATERIALS AND METHODS

The experimental group consisted of transgenic mice models of AD (a model of the formed neurodegenerative changes) mice of the B6SLJ-Tg line (APPSwFlLon,PSEN1*M146L*L286V)6799Vas, males aged 9 months (n = 5). The control group consisted of C57BL/6xSJL mice, males aged 9 months (n = 5). These mouse lines were obtained from The Jackson Laboratory.

In vivo study of BBB permeability was performed by evaluating the permeability of the Evans blue dye in sections of the brain 4–5 hours after its intraperitoneal injection (2% solution in 0.9% NaCl solution, in a volume of 4 ml/kg of animal weight) according to the protocol described [9]. Transcardial perfusion of the brain with 10% formalin was performed to the animals. The Evans blue dye fluorescence area percent of the total area of the vessels in the field of view in the coronal sections of the brain (thickness 50 μm) was calculated using confocal microscopy. Evaluation of the CD31 expression (Abcam, ab28364, rabbit polyclonal, 1: 1000) on free-floating sections was carried out using the standard method of simultaneous combined staining of the drug (free-floating sections staining protocol from Abcam, USA) [10]. The percentage of cells expressing CD31 was calculated (of the total cell number in the vascular region in the field of view, calculated from the nuclei of DAPI-positive cells localized in the vascular region) in three fields of view. Confocal microscopy was performed using an Olympus FV10i-W microscope (Japan). When analyzing photographs, Olympus FLUOVIEW Viewer 4.0 (Japan) was used.

The study of the features of the formation of the vascular network (angiogenesis) was conducted by the microscopic method using a ZOE microscope with photofixation and subsequent processing of the obtained photographs in ImageJ v1.43 (USA). The total length of the vasculature, the number of visible vessels, the number of branch points of the vessels, and the average diameter of the vessels in 1 mm^3 of the hippocampal tissue in the areas CA1, CA2, CA3, dentate gyrus (DG) were calculated.

Statistical analysis of the results was carried out using the Statplus Professional program, assembly 5.9.8.5/Core v.5.9.3.33 using nonparametric statistical methods in GraphPad6.0 program. To compare the performance in independent samples, the Mann – Whitney test was used. Differences were considered significant at $p \leq 0.05$. The results are presented in the form Me [Q_1; Q_3], where Me is the median, Q_1 is the lower quartile, Q_3 is the upper quartile, and p is the significance level.

RESULTS

It was revealed that the total length of the vessels in the dentate gyrus region in transgenic animals is 2.5 times greater than in animals of the control group (p = 0.006) (Fig. 1,*a*). Moreover, the total number of vessels in the CA1 area and the dentate gyrus exceed-

ed the values of the control animals 2.5 and 3 times, respectively ($p = 0.008$) (Fig. 1*b*). However, the number of branch points of blood vessels was significantly higher (1.5–2 times) in the CA1, CA2, CA3 regions in the control animals compared with the animals of the experimental group (Fig. 1,*c*). The mean vessel diameter was statistically significantly smaller in animals with a transgenic model of AD than in animals of the control group in all studied areas of the hippocampus (Fig. 1*d*). Thus, the formation of CAA is accompanied by remodeling of the vascular network of the hippocampus.

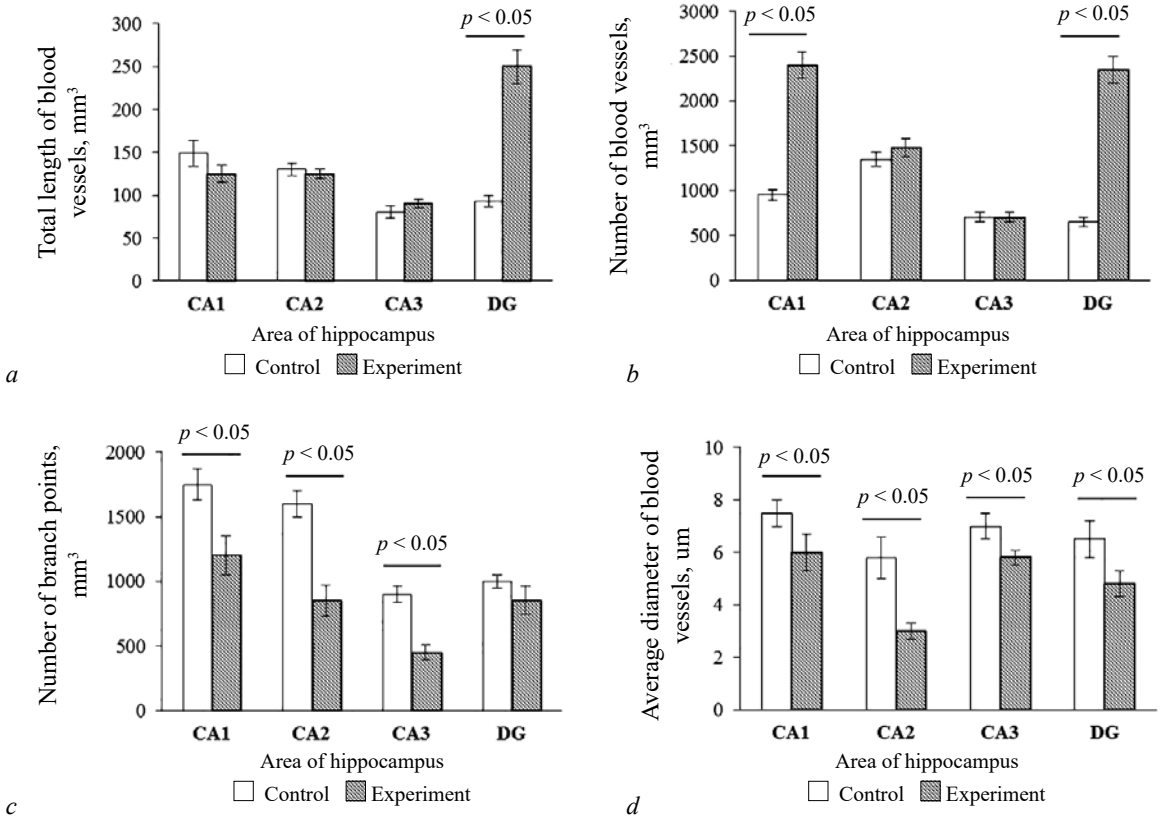

Fig. 1. Features of the vascular network (angiogenesis) in certain areas of the hippocampus in the experimental groups: *a* – total length of blood vessels, mm^3, *b* – number of blood vessels, mm^3, *c* – number of branch points, mm^3, *d* – average diameter of blood vessels; experimental group – animals with a transgenic model of Alzheimer's disease, control group – wild-type animals

We evaluated BBB permeability using Evans blue as a marker for BBB damage. We found that the relative area of brain tissue containing Evans blue dye in the CA2 zone of the hippocampus of transgenic animals is statistically significantly ($p = 0.025$) greater (17.80 [9.15; 36.75]) compared to the control group (1.38 [0.04; 7.60]) (Fig. 2,*a*). A similar statistically significant difference ($p = 0.033$) is also observed in the CA1 zone of the hippocampus (Fig. 2,*a*). The tendency ($p = 0.149$) to increase the relative area containing the dye in the dentate gyrus of the hippocampus is observed in animals of the experimental group (7.37 [1.25; 27.83]) compared with the control group (1.11 [0.05]; 6.35]) (Fig. 2,*a*). A similar situation ($p = 0.157$) was also detected in the CA3 zone of the hippocampus (Fig. 2,*a*).

The results above formed our interest in assessing the expression pattern of one of the endothelial cell markers, namely, CD31, in various hippocampal subregions in animals with a transgenic model of AD. We revealed a tendency ($p = 0.223$) to decrease the number of CD31+ endothelial cells in the dentate gyrus of the hippocampus in animals of the experimental group (21.52 [17.56; 24.50]) compared with the control group (23.08 [21.18; 29.84]) (Fig. 2,*b*). A similar situation is observed in the CA2 and CA3 zones of the hippocampus (Fig. 2,*b*). However, a statistically significant ($p = 0.028$) increase in the number of CD31+ cells in the CA1 hippocampal subregion is observed in animals with a transgenic model of AD (30.41 [20.50; 31.82]) compared with the control group (22.56 [15, 70; 25.34]) (Fig. 2,*b*).

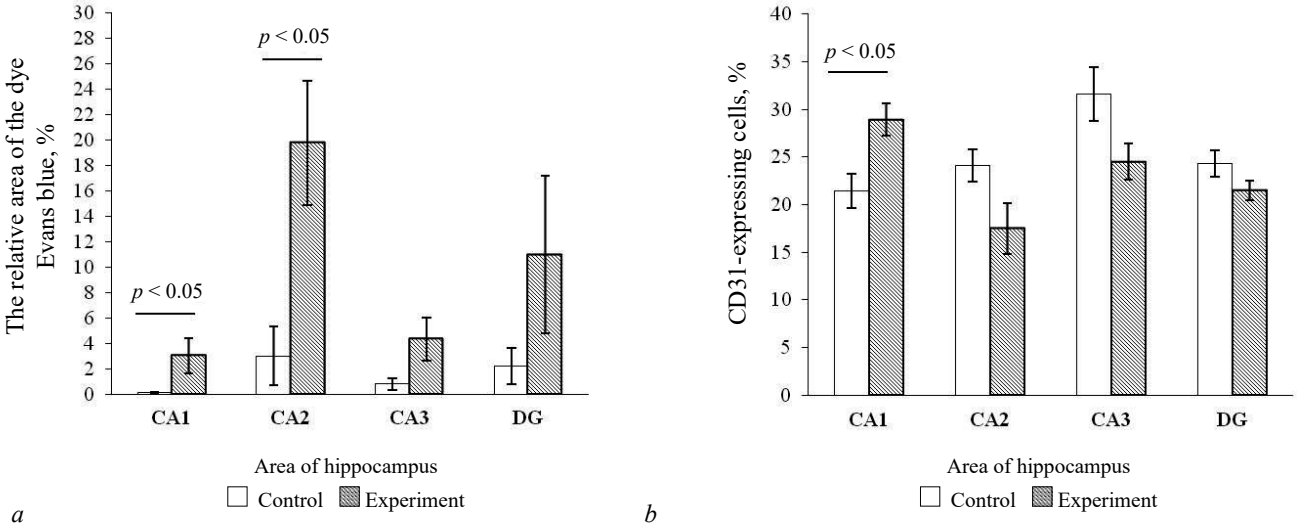

Fig. 2. Permeability of BBB *in vivo* and an expression of CD31: *a* – the amount dye of Evans blue in certain areas of the hippocampus (%) in the experimental groups; *b* – the number of cells expressing CD31 in certain areas of the hippocampus (%) in the experimental groups; experimental group – animals with a transgenic model of Alzheimer's disease, control group – wild-type animals

DISCUSSION

CAA has been shown to play a key role in the pathogenesis of dementia. CAA is most common with the development of a sporadic form of AD, which indicates the presence of a pronounced relationship between AD and cerebral angiopathy. In addition, various microvascular disorders, in particular, decreased capillary density, vascular atrophy, and endothelial dysfunction are observed in the brain with AD progression [11]. It is known that the action of angiogenic growth factors and cytokines in brain tissue leads to the activation of endothelial cells. This promotes the formation of a microvascular network, which increases local microcirculation, inducing the flow of oxygen and nutrients into the affected tissue. The endothelium also has a direct local effect, producing at least 20 paracrine factors that affect neighboring cells. Although many of these factors are anti-apoptotic, toxic substances, including neurotoxins and Aβ precursors, are also released in microvessels of affected tissues [12].

Our study showed significant changes in the microvascular bed of the hippocampus in animals with formed manifestations of chronic neurodegeneration. At the same time, the CA1 subregion of the hippocampus is the most affected area (an increase in the total number of vessels, a decrease in the number of branches of the vasculature, and a decrease in the average diameter of microvessels). Since we found that high levels of CD31 cells remained in precisely this hippocampal subregion, it is logical to assume that the CA1 zone and the dentate gyrus of the hippocampus dominate in the process of the microvascular network remodeling during Alzheimer's type neurodegeneration. Thus, the processes of the microvascular bed remodeling are multidirectional in the hippocampal subregions in animals with experimental AD: neoangiogenesis is typical for the CA1 zone of the hippocampus and the dentate gyrus, and local microcirculation disorders due to the reduction in the diameter and branching of the vessels is typical for the CA2 and CA3 subregions.

These results are consistent with the data on the intensification of the brain neoangiogenesis in experimental animals during Alzheimer's neurodegeneration [13]. This may be accompanied by the development of pathological BBB permeability due to the impaired expression of tight junction proteins of cerebral endothelial cells. At the microvascular level, endothelial cell and BBB dysfunction may be associated with decreased cerebral blood flow and hypoxia. In addition, Aβ concentration in microvessels can contribute to further damage to endothelial cells, which may be one of the links in the AD pathogenesis [14]. An impaired, due to endothelial dysfunction, Aβ drainage can lead to the accumulation of amyloid plaques in the brain parenchyma. Experimental data show [15] that the action of Aβ on proteins of tight and adhesive junctions can change the BBB permeability. Damage to the BBB leads to the death of neuronal cells, glia acti-

vation, and immune infiltration into the parenchyma. This has negative consequences for the affected areas of the brain: hormonal dysregulation in hypothalamic lesions [16], cognitive impairment in hippocampal lesions [17], which contribute to the progression of AD.

Thus, our results indicate that an increase in BBB permeability is most characteristic of the CA2 subregion of the hippocampus, to a lesser extent, the CA1 sub-region, and, most likely, the dentate gyrus of the hippocampus. A significant increase in BBB permeability may be associated with intensive neoangiogenesis, microvessel remodeling (decreased branching of vessels and a reduction in mean vessel diameter, and an increased expression of CD31 in the CA1 hippocampal subregion) against the background of the development of CAA.

CONCLUSION

It was found that neurodegenerative changes in the hippocampus of transgenic mice related to the Aβ accumulation are associated with the local disturbance of microcirculation. This is a consequence of a reduction in the diameter and branching of blood vessels, an increase in BBB permeability, and suppression of neoangiogenesis (except the CA1 subregion) as the disease progresses. These observations emphasize the importance of future research for a clear understanding of the molecular mechanisms of cerebral microcirculatory disorders and a violation of the structural and functional integrity of the BBB in chronic Alzheimer's disease neurodegeneration.

REFERENCES

1. Kumfor F., Piguet O. Emotion recognition in the dementias: brain correlates and patient implications. *Neurodegener Dis. Manag.* 2013; 3 (3): 277–288. DOI: 10.2217/nmt.13.16.
2. Chui H.C., Ramirez-Gomez L. Clinical and imaging features of mixed Alzheimer and vascular pathologies. *Alzheimers Res. Ther.* 2015; 7 (1): 21. DOI: 10.1186/s13195-015-0104-7.
3. Kalaria R.N. Small vessel disease and Alzheimer's dementia: pathological considerations. *Cerebrovasc Dis.* 2002; 13(2): 48–52. DOI: 10.1159/000049150.
4. Goos J.D.C., Kester M.I., Barkhof F., Klein M., Blankenstein M.A., Scheltens P. van der Flier W.M. Patients with Alzheimer disease with multiple microbleeds. *Stroke.* 2009; 40 (11): 3455–3460. DOI: 10.1161/STROKEAHA.109.558197.
5. Garcia-Alloza M., Gregory J., Kuchibhotla K.V., Fine S., Wei Y., Ayata C., Frosch M.P., Greenberg S.M., Bacskai B.J. Cerebrovascular lesions induce transient β-amyloid deposition. *Brain.* 2011; 134 (12): 3694–3704. DOI: 10.1093/brain/awr300.
6. Uryu K., Laurer H., McIntosh T., Praticò D., Martinez D., Leight S., Lee V.M., Trojanowski J.Q. Repetitive mild brain trauma accelerates Aβ deposition, lipid peroxidation, and cognitive impairment in a transgenic mouse model of alzheimer amyloidosis. *J. Neurosci.* 2002; 22 (2): 446–454. DOI: 10.1523/JNEUROSCI.22-02-00446.2002.
7. Sengillo J.D., Winkler E.A., Walker C.T., Sullivan J.S., Johnson M., Zlokovic B.V. Deficiency in mural vascular cells coincides with blood-brain barrier disruption in Alzheimer's disease. *Brain Pathol.* 2013; 23 (3): 303–310. DOI: 10.1111/bpa.12004.
8. Montagne A., Barnes S.R., Sweeney M.D., Halliday M.R., Sagare A.P., Zhao Z., Toga A.W., Jacobs R.E., Liu C.Y., Amezcua L., Harrington M.G., Chui H.C., Law M., Zlokovic B.V. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron.* 2015; 85 (2): 296–302. DOI: 10.1016/j.neuron.2014.12.032.
9. Manaenko A., Chen H., Kammer J., Zhang J. H., Tang J. Comparison evans blue injection routes: intravenous versus intraperitoneal, for measurement of blood-brain barrier in a mice hemorrhage model. *J. Neurosci. Methods.* 2011; 195 (2): 206–210. DOI: 10.1016/j.jneumeth.2010.12.013.
10. Encinas J.M., Enikolopov G. Identifying and quantitating neural stem and progenitor cells in the adult brain. *Methods Cell Biol.* 2008; 85: 243–272. DOI: 10.1016/S0091-679X(08)85011-X.
11. Kalaria R.N. Neuropathological diagnosis of vascular cognitive impairment and vascular dementia with implications for Alzheimer's disease. *Acta Neuropathol.* 2016; 131 (5): 659–685. DOI: 10.1007/s00401-016-1571-z.
12. Grammas P., Moore P., Weigel P.H. Microvessels from Alzheimer'sdisease brains kill neurons in vitro. *Am. J. Pathol.* 1999; 154 (2): 337–342. DOI: 10.1016/S0002-9440(10)65280-7.
13. Biron K.E., Dickstein D.L., Gopaul R., Jefferies W.A. Amyloid triggers extensive cerebral angiogenesis causing blood brain barrier permeability and hypervascularity in Alzheimer's disease. *PLoS One.* 2011; 6 (8): e23789. DOI: 10.1371/journal.pone.0023789.
14. Burgmans S., van de Haar H. J., Verhey F. R. J., Backes W. H. Amyloid-β interacts with blood-brain barrier function in dementia: a systematic review. *J. Alzheimers Dis.* 2013; 35 (4): 859–873. DOI: 10.3233/JAD-122155.
15. Marco S., Skaper S.D. Amyloid beta-peptide1-42 alters tight junction protein distribution and expression in brain microvessel endothelial cells. *Neurosci. Lett.* 2006; 401 (3): 219–224. DOI: 10.1016/j.neulet.2006.03.047.
16. Jais A., Brüning J.C. Hypothalamic inflammation in obesity and metabolic disease. *J. Clin. Invest.* 2017; 127 (1): 24–32. DOI: 10.1172/JCI88878.
17. Davidson T.L., Monnot A., Neal A.U., Martin A.A., Horton J.J., Zheng W. The effects of a high-energy diet on hippocampal-dependent discrimination performance and blood-brain barrier integrity differ for diet-induced obese and diet-resistant rats. *Physiol. Behav.* 2012; 107 (1): 26–33. DOI: 10.1016/j.physbeh.2012.05.015..

Authors contribution

Gorina Ya.V. – analysis and interpretation of immunohistochemical data. Osipova E.D. – assessment of the features of the formation of the vascular network (angiogenesis). Morgun A.V. – analysis and interpretation of angiogenesis research data. Malinovskaya N.A. – assessment of BBB permeability. Komleva Yu.K. – conception and design. Lopatina O.L. – design drawings. Salmina A.B. – critical revision for important intellectual content and final approval of the manuscript for publication.

Authors information

Gorina Yana V., Cand. Sci. (Pharmacy), Associate Professor, Department of Biological Chemistry, Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation. ORCID 0000-0002-3341-1557.

Osipova Elena D., Senior Teacher, Department of Biological Chemistry, Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation. ORCID 0000-0002-9718-1260.

Morgun Andrei V., Dr. Sci. (Med.), Assistant, Pediatrics Department of the Institute of Post-graduate Education, Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation. ORCID 0000-0002-9644-5500.

Malinovskaya Nataliya A., Dr. Sci. (Med.), Professor, Department of Biological Chemistry of Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation. ORCID 0000-0002-0033-3804.

Komleva Yuliya K., Cand. Sci. (Med.), Associate Professor, Department of Biological Chemistry, Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation. ORCID 0000-0001-5742-8356.

Lopatina Olga L., Dr. Sci. (Biology), Professor, Department of Biological Chemistry, Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation. ORCID 0000-0002-7884-2721.

Salmina Alla B., Dr. Sci. (Med.), Professor, Head of the Department of Biological Chemistry, Head of the Research Institute of Molecular Medicine and Pathological Biochemistry, Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation. ORCID 0000-0002-9170-0867.

(✉) **Gorina Yana V.,** e-mail: yana_20@bk.ru.

Received 25.08.2019
Accepted 25.12.2019