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EXPERIMENTAL ARTICLES

The Endothelium-Protective Effect of 3-Methyl-1,2,4-triazolyl-5-thioacetate (S)-2,6-diaminohexanic Acid (Lysinium): Effects on the Expression of Vascular Endothelial Growth Factor (VEGF) and the Characteristics of the Endotheliocytes of the Cerebral Vessels of Animals with Cerebral Ischemia

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Abstract—We modeled acute disruption of the brain blood circulation (ADBC) by bilateral ligature of the common carotids in white outbred rats. This resulted in the formation of endothelial dysfunction of capillary network in the IV–V layers of the cortex and the vessels of the pia mater, choroids plexuses, and the branches of the middle cerebral and ophthalmic arteries, which was seen as a considerable decrease in the density of nuclei of endotheliocytes, a decrease in the RNA concentration in them, expression of vascular endothelial growth factor (VEGF), and a substantial decrease in the index of the proliferative activity of endotheliocytes. Intraperitoneal treatment of rats with ADBC with lysinium for 21 days at a dose of 50 mg/kg led on the 4th day to an increase in the density of endotheliocytes in the capillary network of the brain cortex and the walls of cerebral vessels and an increase in the RNA content in endothelial nuclei. At the end of the lysinium treatment, we observed an increase in the density of endotheliocytes in the capillary network of the brain cortex and the walls of cerebral vessels, an increase in the RNA content in nuclei of endotheliocytes, and an increase in the density of proliferating endotheliocytes in these vessels, which was associated with increased VEGF concentration. Administration of the control drug piracetam had no endothelium-protective effect.

Keywords: cerebral ischemia, morpho-functional indices of endotheliocytes, vascular endothelial growth factor (VEGF), endothelium-protective effect, 3-methyl-1,2,4-triazolyl-5-thioacetate (S)-2,6-diaminohexanic acid, lysinium

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INTRODUCTION

Currently, acute disruption of brain blood circulation (ADBC) is the second worldwide cause of disability and the third cause of death of people in industrially developed countries [1–5]. It has been shown that polymorphism of ADBC is determined by different pathogenetic mechanisms, such as atherothrombosis, cerebral embolism, cardiogenic disruptions of systemic hemodynamics, and alterations in small cerebral vessels during arterial hypertension [6, 7]. Currently, the vascular endothelium, which is considered both as a target for brain insult and as an effector in the pathogenesis of these diseases [2, 3, 5, 8], is an object of intense attention of neurophysiologists, pharmacologists, and clinicians. Recently, the term endothelial dysfunction (ED) has appeared; it includes endothelial structural and functional changes, such as inadequate formation of different biologically active substances in the endothelium [2, 5]. ED is considered as a predictor of brain damage during cerebrovascular diseases [5]. An important factor that influences ADBC outcome is the organization of timely help to patients with this disease [5, 8]. Starting from the first minutes of an ischemic insult, an important role is played by endothelium-protective therapy if we consider the initial deficit of local brain circulation followed by the progression of neurodestruction as processes in which vessels actively participate [1, 2, 5, 8]. However, currently there are no drugs with proven endothelium-protective action. We believe that future drugs will have not only neuroprotective action but also an indirect positive effect on endothelial function. Therefore, an urgent task of modern neuropharmacology is the search for drugs that have strong endothelium-protective, antioxidant, and NO-modulating effects under conditions of cerebrovascular pathology [1, 2, 5, 8].

The L-lysine-based drug aescinat, which has anti-inflammatory and antiexudative characteristics and

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increases vascular tone, is more widely used in angioneurology [2, 3, 8, 9].

The means of secondary neuroprotection include antioxidants and neurometabolic cerebroprotective drugs (emoxipine, mildronate, piracetam, and thiotriazoline), which under experimental conditions improve neurochemical processes, inhibit oxidative modification of protein fragments of receptors and ion channels, and protect membranes [1, 8, 10–13, 16, 17]. Thiotriazoline (3-methyl-1,2,4,-triazolyl-5-thioacetate morpholinium), a drug from this group, which regulates brain blood circulation at the level of large and moderate vessels, maintains the permeability of microcirculation, protects NO from reactive oxygen species, and increases its biological availability, may have endothelium-protective characteristics [8, 11, 17].

To create an effective endothelium-protective drug, the NPO Farmakon synthesized 3-methyl-1,2,4-triazolyl-5-thioacetate (S)-2,6-diaminohexanic acid (Lysinium), whose structure is a combination of fragments of molecules of thiotriazoline and L-lysine aescinate. This drug has antiischemic, cardioprotecive, neuroprotective, antioxidant, and anti-inflammatory activities and its efficacy is higher than the efficacy of its structural "parents" [9, 13, 14]. It has been shown that administration of lysinium to animals with ischemia of the myocardium resulted in normalization of the ratio of the thiol-disulfide system and NO in the myocardium and improved the indices of cardiac hemodynamics. As well, lysinium increased the viability of neurons of the sensomotor cortex, increased RNA content in their nuclei, and decreased the number of neurons with signs of apoptosis and neurodegeneration under conditions of cerebral ischemia [15].

The purpose of our study was to evaluate the endothelium-protective effect of the potential medication lysinium and validate its use in the pathology of the cardiovascular system on the basis of data on the peculiarities of dysfunction of the endothelium of cerebral vessels in a model of acute disruption of brain circulation.

MATERIALS AND METHODS

We used white Wistar rats of both sexes weighing 180–200 g, which were obtained from the State Institute of Pharmacology and Toxicology of the Ukrainian Academy of Medical Sciences (Kiev). The duration of the quarantine (acclimatization period) for all animals was 14 days. During the quarantine, we performed daily examination of each animal (behavior and general state) and twice a day inspected cages for death and morbidity. Before the start of the experiments, animals that corresponded to criteria of inclusion in the experiment were randomly subdivided in groups. Animals that did not correspond to the criteria were excluded from the study during the quarantine. Animals were kept under standard conditions of a vivarium with free access to water and standard granulated feed.

All the experimental procedures were performed in accordance with "Regulations on the use of animals in biomedical studies." In our study, our goal was to evaluate the endothelium-protective activity of the new substance under conditions of cerebral ischemia; hence, we needed experimental models of pathology of the brain that are adequate to clinical situations, such as ischemic insult. These models include uni- and bilateral ligature of the common carotids and temporal occlusion of two carotids accompanied by irreversible ligature of two vertebral arteries. Bilateral ligature of the common carotids corresponds to our criteria and species-specific anatomical–physiological characteristics of brain blood circulation of white rats. This procedure leads to a typical neurological deficit, cognitive disorders, and histological and biochemical changes in the brain tissue [16].

The hair at the surgical site was shaven and the surgical area was treated with an antiseptic. The experiment was performed in an experimental operating room after UV illumination and treatment with antiseptics at a temperature of 19–20°C. Bilateral ligature of the common carotids was performed under thiopental narcosis (40 mg/kg); the carotids were surgically separated and simultaneously ligatured by a silk ligature. After occlusion of carotids, the wounds were stitched layer-by-layer and treated with brilliant green. In sham-operated animals, we made an incision and stitched the skin. The development of endothelium dysfunction and endothelium-protective activity of lysinium was evaluated by the morphofunctional characteristics of endotheliocytes in the capillary network of the brain cortex and the walls of cerebral vessels in the acute (4th day) and recovery (21st day) periods of experimental disruption of the brain blood circulation. Animals of different groups were anesthetized by thiopental (40 mg/kg) on the 4th and 21st days. Intraperitoneal injections were made for 4 and 21 days starting immediately after arousal of animals after anesthesia. Lysinium (2.5% solution for injections (NPO Farmakon, Ukraine)) was injected at a dose of 50 mg/kg (2 mL/kg). As a reference drug, we used the neurometabolic cerebroprotective drug piracetam, which is widely used in neurological practice [17]. We used 20% solution of piracetam for injections in 5 mL ampoules (Sanitas, Latvia) at a dose 250 mg/kg (1.25 mL/kg) [17]. The control group (uninjected animals) was treated with 2 mL/kg of physiological solution.

In total, we used 70 animals, which were subdivided into seven groups:

1. Sham-operated, 10 rats;

2. Control (animals with occlusion of the common carotids that were treated with physiological saline for 4 days), 10 rats;

3. Animals with occlusion of the common carotids that were treated with lysinium for 4 days, 10 rats;

4. Animals with occlusion of the common carotids that were treated with piracetam for 4 days, 10 rats;

5. Control (animals with occlusion of the common carotids that were treated with physiological saline for 21 days), 10 rats;

6. Animals with occlusion of the common carotids that were treated with lysinium for 21 days, 10 rats;

7. Animals with occlusion of the common carotids that were treated with piracetam for 21 days, 10 rats.

Immunohistochemical Methods

At day 21, the animals were decapitated under sodium thiopental anesthesia (40 mg/kg) and their brains were removed. The brain was fixed in 10% Bouin's liquid (24 h) and, according to a standard scheme, embedded in paraffin blocks, which were used to prepare serial frontal 5 μm histological slices [14]. To study the morphofunctional state of the endotheliocytes of capillaries of cortical layers IV–V and walls of the pia mater, choroids plexuses, branches of the middle cerebral and ophthalmic arteries (referred to below as cerebral vessels), histological slices were deparaffinized according to the standard method [14] and stained with gallocyanin-chrome alums according to Einarson for specific development of RNA. We measured the following indices for endotheliocytes of vessels [16]:

—The nuclear area;

—The mean nuclear diameter, which, taking the fact into account that the shape of the common carotid nucleus of the endothelial cell of vessels is strongly elliptic, was taken as the smallest ellipse diameter;

—The RNA concentration in the nucleus (units of optical density, E_{OD}), which was calculated as the logarithm of the ratio between the optical density of the nucleus to the optical density of the intercellular matrix;

—The density of the nuclei of endotheliocytes per 1 mm^2 of the area of the IV–V cortical layers or the walls of the cerebral vessels.

To determine proliferating endotheliocytes, histological slices of the brain were deparaffinized and rehydrated, washed three times for 5 min in phosphate buffer (pH = 7.4) and incubated for 30 min in 2 N hydrochloric acid at 37°C. Slices were then washed twice for 5 min with a phosphate buffer (pH = 7.4), twice for 5 min with Holmes' borate buffer (pH = 8.4), and four times for 5 min with a phosphate buffer (pH = 7.4); after this, the slices were incubated for 30 min with 0.1% solution of trypsin in a phosphate buffer at 37°C. After incubation, the slices were washed four times for 5 min with phosphate buffer (pH = 7.4) and then incubated for 24 h in a humidified chamber at 4–6°C with monoclonal mouse antibodies to 5-bromo-2'-deoxyuridine (anti-BrdU, clone BU-33; Sigma-Aldrich, cat. no. B8434). After incubation, the slices were washed four times for 5 min with a phosphate buffer (pH = 7.4) and then for 1 h at 37°C with secondary sheep antibodies to the $F_{(AB')2}$ fragment of mouse IgG conjugated with FITC (Sigma-Aldrich, cat. no. F2266). After a final quadruple wash with a phosphate buffer (pH = 7.4), the slices were placed in a mixture of glycerin-phosphate buffer (9 : 1).

Analysis of the histological slices was performed on an Axioskop microscope (Zeiss, Germany) in ultraviolet light. To obtain fluorescent images of the nuclei of endotheliocytes, we used a 38HE high-emission filter (Zeiss, cat. no. 489038-0000) with excitation at 450–490 nm, emission at 500–550 nm, and a Fluar 40x/1.3 specialized objective (Zeiss, cat. no. 440260–9900). Images were obtained using a COHU-4922 8-bit CCD camera (COHU Inc., United States) and a VIDAS-386 system for image analysis (Kontron Elektronik, Germany) and were automatically processed using a macro developed in VIDAS-2.5 software (Kontron Elektronik, Germany).

The expression of vascular endothelial growth factor (VEGF) was measured using primary mouse IgG1 antibodies to endothelial growth factor (clone CH-10) (Chemicon, cat. no. MAB1665). We calculated the VEGF concentration in the tissue we studied (units of optical density, E_{IF}) as a logarithm of the ratio between the fluorescence intensity to the fluorescence of the intercellular matrix [16].

Statistical Methods

The normality of the distribution was analyzed using the Kolmogorov-Smirnov (D), Lilliefors, and Shapiro-Wilk (W) criteria; the last criterion was used more frequently. The data are shown as the mean and the standard error of mean.

The results were analyzed using STATISTICA for Windows 6.0 (StatSoft Inc), SPSS 16.0, and Microsoft Excel 2003. Statistical procedures and algorithms were written as macros in their respective programs. For all types of analysis, the differences were considered as significant at $p < 0.05$. We used the Mann–Whitney criterion.

RESULTS AND DISCUSSION

During the acute period of the modeling of cerebral ischemia (on the 4th day after ligature of the common carotids), we observed a considerable decrease in the density of nuclei of capillary endotheliocytes by 32.3% ($p < 0.05$) per slice area as compared to the sham-operated animals (Table 1). This reflects the formation of ischemia of the brain cortex and endothelial dysfunction, closure of part of the capillaries, and a decrease in the density of functioning capillaries. During the recovery period (the 21st day), we observed partial recovery of the capillary network due to revascularization of ischemic regions. Nevertheless, the density of the nuclei of endotheliocytes at this time remained smaller than in sham-operated rats by 28.6% ($p < 0.05$).

Table 1. The characteristics of the endotheliocytes of the capillaries of cortical layers IV–V of rats with experimental cerebral ischemia, $M \pm m$

Experimental groups	Density of nuclei per 1 mm^2 of the cortex	Area of nuclei, μm^2	Diameter of nuclei, μm	RNA concentration in nuclei, E_{OD}
Sham-operated ($n = 10$)	887 ± 14	8.34 ± 0.07	2.77 ± 0.01	0.293 ± 0.002
Ischemia, day 4 ($n = 10$)	600 ± 11	8.38 ± 0.06	3.07 ± 0.01	0.212 ± 0.001
Ischemia, day 4 + 50 mg/kg lysinium ($n = 10$)	727 ± 10*# (+21.2%)	9.87 ± 0.03*# (+17.8%)	3.11 ± 0.01	0.315 ± 0.001*# (+48.6%)
Ischemia, day 4 + 250 mg/kg piracetam ($n = 10$)	608 ± 15	8.40 ± 0.07	3.06 ± 0.01	0.217 ± 0.001
Ischemia, day 21 ($n = 10$)	633 ± 15	9.11 ± 0.03	3.05 ± 0.01	0.268 ± 0.001
Ischemia, day 21 + 50 mg/kg lysinium ($n = 10$)	837 ± 17*# (+32%)	8.38 ± 0.03* (−8%)	2.93 ± 0.01*	0.298 ± 0.002*# (+11.2%)
Ischemia, day 21 + 250 mg/kg piracetam ($n = 10$)	650 ± 15 (+2.7%)	9.05 ± 0.05	3.02 ± 0.01	0.273 ± 0.001 (+1.8%)

Note: %, as compared to groups of untreated animals with ischemia at days 4 and 21, respectively; *, as compared to the control group on the respective day (Mann–Whitney U-criterion, $p < 0.05$); #, compared to the piracetam group on the respective day (Mann–Whitney U-criterion, $p < 0.05$).

Table 2. The characteristics of proliferating endotheliocytes of the capillaries of cortical layers IV–V of rats with experimental cerebral ischemia, $M \pm m$

Experimental groups	Density of proliferating cells per 1 mm^2 of the cortex	Area of nuclei, μm^2	Diameter of nuclei, μm	VEGF concentration, E_{IF}
Sham-operated ($n = 10$)	771 ± 26	6.77 ± 0.10	2.88 ± 0.02	0.78 ± 0.01
Ischemia, day 21 ($n = 10$)	474 ± 22	7.41 ± 0.10	2.92 ± 0.02	0.81 ± 0.02
Ischemia, day 21 + 50 mg/kg lysinium ($n = 10$)	637 ± 21*# (+34.4%)	8.12 ± 0.09 *# (+9.6%)	3.18 ± 0.01*# (+9%)	0.98 ± 0.02*# (+21%)
Ischemia, day 21 + 250 mg/kg piracetam ($n = 10$)	477 ± 23	7.43 ± 0.11	3.00 ± 0.02	0.86 ± 0.02

Note: %, as compared to groups of untreated animals with ischemia on day 21; *, as compared to the control group on the respective day (Mann–Whitney U-criterion, $p < 0.05$); #, compared to the piracetam group at respective day (Mann–Whitney U-criterion, $p < 0.05$).

Modeling of ADBC was associated with a decrease in the RNA concentration in the nuclei of the endotheliocytes of capillaries by 27.6% ($p < 0.05$), as compared to the sham-operated animals on the 4th day after ADBC and partial recovery of the RNA pool to the 21st day (an increase by 26.4% as compared to this index on the 4th day of ischemia) (Table 1).

Administration of lysinium protected the endoliocytes of capillaries already by the 4th day, which was seen as a significant increase in the density of the nuclei of endotheliocytes (21%), the hypertrophy of nuclei (17%), and an increase in RNA concentration by 48% as compared to analogous indices of untreated animals. The strongest effect of lysinium was observed on the 21st day, which was seen as practically complete recovery of the density of capillary endotheliocytes and an increase in the RNA concentration level of the sham-operated animals. This fact may reflect the complete revascularization and recovery of the capillary network in the brain cortex of ischemic animals treated with lysinium. Treatment of animals with ADBC with the reference drug piracetam at a dose of 250 mg/kg did not protect the endothelium of cortical capillaries.

For a more detailed description of the revascularization, we studied the degree of VEGF binding and the character of the proliferative activity of the capillary endothelium for 10 days (Table 2).

We found that at the 21st day after ligature of common carotids, VEGF binding with vascular endothelium of the capillary network of the cortex was significantly increased as compared to the sham-operated animals. This fact does not contradict the data of other researchers and our previous works [3, 8, 12, 18],

Table 3. The characteristics of the endotheliocytes of the walls of cerebral vessels of rats with experimental cerebral ischemia, $M \pm m$

Experimental groups	Density of nuclei per 1 mm^2 of vessel wall	Area of nuclei, μm^2	Diameter of nuclei, μm	RNA concentration in nuclei, E_{OD}
Sham-operated ($n = 10$)	14168 ± 553	7.04 ± 0.26	2.94 ± 0.05	0.294 ± 0.003
Ischemia, day 4 ($n = 10$)	9938 ± 373	8.00 ± 0.25	3.18 ± 0.04	0.231 ± 0.002
Ischemia, day 4 + 50 mg/kg lysinium	12565 ± 311*# (+21%)	8.14 ± 0.23	3.12 ± 0.03	0.310 ± 0.001*# (+34.2%)
Ischemia, day 4 + 250 mg/kg piracetam ($n = 10$)	9939 ± 340	6.20 ± 0.20	3.17 ± 0.03	0.237 ± 0.001
Ischemia, day 21 ($n = 10$)	11378 ± 281	5.43 ± 0.11	2.71 ± 0.03	0.247 ± 0.001
Ischemia, day 21 + 50 mg/kg lysinium ($n = 10$)	13987 ± 411*# (+23%)	8.05 ± 0.29 *# (+48.3%)	3.14 ± 0.05*#	0.317 ± 0.003*# (+28.3%)
Ischemia, day 21 + 250 mg/kg piracetam ($n = 10$)	11487 ± 267	5.87 ± 0.21	2.80 ± 0.02	0.248 ± 0.001

Note: %, as compared to groups of untreated animals with ischemia at days 4 and 21, respectively; *, as compared to the control group on the respective day (Mann–Whitney U-criterion, $p < 0.05$); #, compared to the piracetam group on the respective day (Man–Whitney U-criterion, $p < 0.05$).

which showed that during the acute period of myocardial infarction or brain insult VEGF expression is suppressed and during the recovery period its expression was somewhat increased due to the recovery of the collateral blood circulation. During the recovery period after a brain insult, VEGF induces the growth of a number of new vessels and suppresses the inflammatory reaction and its deficit points to the formation of endothelial dysfunction. These results suggest that disruption of the systemic expression of VEGF may be one of the etiological factors of the development of endothelial dysfunction. Administration of lysinium positively influenced this process, which is supported by an increase by 21% in the VEGF concentration as compared to untreated animals on the 21st day. Ischemia suppressed the proliferative activity of the vascular endothelium of capillaries in the brain cortex, which was seen as a decrease by 46% in the density of proliferating cells per 1 mm^2 of the cortex ($p < 0.05$) as compared to the sham-operated animals. Treatment of animals with ADBC with lysinium substantially increased the proliferative activity of the vascular endothelium, which is supported by a significant increase in the density of proliferating endotheliocytes by 34% and the diameter of the nuclei of proliferating cells as compared to untreated animals.

The data we obtained suggest that lysinium indirectly via an increase in VEGF expression or independently considerably increased the proliferative activity of the endothelium in capillaries of the brain cortex, which promotes effective revascularization of ischemic nervous tissue.

Analysis of the walls of the vessels of the pia mater, choroids plexuses, and the branches of the middle cerebral and ophthalmic arteries showed that the density of the nuclei of endotheliocytes here exceeds this index in the capillary network of the brain cortex. At day 4, we observed a decrease in the density of endotheliocytic nuclei by 29.8% ($p < 0.05$) per area unit and a decrease in RNA concentration by 21.4% ($p < 0.05$) as compared to the sham-operated animals (Table 3).

This points to damage of vessels of a muscular type in the ischemic zone, which inevitably influences the functional state of the microcirculation of the brain and the formation of endothelial dysfunction [18].

However, at day 21, the density of the nuclei of endotheliocytes in the vascular walls gradually increased but remained smaller by 19.7% ($p < 0.05$) than in sham-operated rats. We observed a decrease in the RNA pool in the nuclei of endotheliocytes of the vascular walls on the 4th day; by the 21st day after ADBC the RNA deficit in the nuclei of brain vascular endotheliocytes was 16% ($p < 0.05$) as compared to the indices of the sham-operated animals (Table 3).

Analysis of the binding of vascular endothelial growth factor (VEGF) and character of proliferative activity of vascular endothelium of the brain showed that ischemic brain damage resulted in an increase in VEGF binding with the endothelium of the cerebral vessels as compared to the sham-operated animals by 2.5 times at the 21st day of experiment. Administration of lysinium to animals with ADBC for 21 days intensified VEGF binding with the vascular endothelium by 40% as compared to the control group. Administration of piracetam did not affect VEGF binding with the brain vascular endothelium. ADBC suppressed the proliferative activity of the vascular endothelium of the brain. The density of the prolifer-

Table 4. The characteristics of the proliferating endotheliocytes of cerebral vessels of rats with experimental cerebral ischemia, $M \pm m$

Experimental groups	Density of nuclei per 1 mm^2 of the cortex	Area of nuclei, μm^2	Diameter of nuclei, μm	VEGF concentration, E_{IF}
Sham-operated ($n = 10$)	8430 ± 893	14.78 ± 0.86	3.82 ± 0.12	1.34 ± 0.08
Ischemia, day 21 ($n = 10$)	2882 ± 300	6.68 ± 0.46	2.81 ± 0.1	3.00 ± 0.18
Ischemia, day 21 + 50 mg/kg lysinium ($n = 10$)	4115 ± 277*# (+42.8%)	14.12 ± 0.77*# (+111.4%)	3.67 ± 0.07*# (+30.6%)	4.18 ± 0.31*# (+40%)
Ischemia, day 21 + 250 mg/kg piracetam ($n = 10$)	2698 ± 277	6.70 ± 0.44	2.82 ± 0.1	3.00 ± 0.11

Note: %, as compared to groups of untreated animals with ischemia at day 21; *, as compared to the control group on the respective day (Mann–Whitney U-criterion, $p < 0.05$); #, compared to the piracetam group on the respective day (Mann–Whitney U-criterion, $p < 0.05$).

ating cells by the 21st day decreased by 65% (Table 4) as compared to the sham-operated animals ($p < 0.05$).

Administration of lysinium significantly increased the density of proliferating endotheliocytes in cerebral vessels by 42.8%. Lysinium also increased the area of the nuclei by 111% and the diameter of the nuclei by 30% (Table 4). These data point to a strong endothelium-protective action of lysinium.

Thus, modeling of ischemic insult results in the formation of endothelial dysfunction, which is seen as a decrease in the density of the nuclei of endothelyocytes of cerebral vessels and the capillary network of the brain cortex, a significant increase in the degree of binding of vascular endothelium growth factor (VEGF) with vessel walls, and a decrease in the number of proliferating endotheliocytes and the index of their proliferative activity. The strongest endothelial dysfunction was found during the acute period of cerebral ischemia (day 4) and maintained for the entire period of observations (day 21).

A course of lysinium for 21 days to animals with ADBC resulted in an increase in the density of the endotheliocytes of the cortical capillary network and the walls of cerebral vessels, an increase in the RNA pool, and activation of translational activity of cells from the 4th day of the experiment, which, finally, led to a complete recovery of the density of endotheliocytes by the 21st day. A course of treatment with lysinium of animals with ADBC resulted in an increase in the proliferating activity of the vascular endothelium in the cerebral vessels and the cortical capillary network and an increased index of their proliferation due to either influence on the VEGF or independent action on the endothelium.

CONCLUSIONS

(1) Modeling of ADBC by bilateral occlusion of the common carotids resulted in a considerable decrease in the density of the nuclei of endotheliocytes and a decrease in RNA concentration in them in the capillary network of cortical layers IV–V, vessels of the pia mater and choroids plexuses, as well as the branches of the middle cerebral and ophthalmic arteries.

(2) The strongest alterations in morphofunctional indices of endotheliocytes were recorded during the acute period of cerebral ischemia (4th day) with maintenance of these disruptions until the 21st day.

(3) Modeling of ADBC was accompanied by the expression of VEGF and considerable suppression of proliferative activity of endotheliocytes at day 21.

(4) Intraperitoneal treatment of animals with ADBC with lysinium at a dose of 50 mg/kg for 21 days led to an increase in the density of the nuclei of endotheliocytes of the capillary network of the brain cortex and the walls of cerebral vessels, an increased RNA content in them, an increase in the proliferative activity of the cells of the vascular endothelium, and an increase in their proliferation index due to VEGF expression.

(5) Intraperitoneal treatment of animals with ADBC with piracetam at a dose of 250 mg/kg for 21 days had no significant influence on morphofunctional indices of endotheliocytes.

(6) The data we obtained suggest that lysinium has a pronounced endothelium-protective effect.

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