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Регуляция функционирования ABCB1-белка в коре головного мозга на фоне глобальной церебральной ишемии

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АННОТАЦИЯ

Введение. ABCB1-белок — мембранный транспортер, осуществляющий эффлюкс из клеток широкого спектра лекарственных веществ. Изучение механизмов регуляции функционирования ABCB1-белка в головном мозге на фоне его ишемии позволит предложить новые подходы к фармакотерапии церебральной ишемической патологии.

Цель. Изучить регуляцию функционирования ABCB1-белка в коре головного мозга крыс при глобальной церебральной ишемии.

Материалы и методы. Эксперимент выполнен на 30 крысах-самцах, которым моделировали глобальную церебральную ишемию путем билатеральной окклюзии общих сонных артерий. В коре головного мозга методом иммуноферментного анализа определяли количество ABCB1-белка и транскрипционных факторов Nrf2 и HIF-1α. Свободнорадикальный статус коры больших полушарий оценивали по концентрации малонового диальдегида, SH-групп, активности глутатионпероксидазы (G-per).

Результаты. Билатеральная окклюзия общих сонных артерий вызывала увеличение уровня ABCB1-белка в коре головного мозга крыс к четвертому часу ишемии, через 24 ч. его количество оставалось повышенным, а через 72 ч. уменьшалось до значений, не отличающихся от показателей ложнооперированных крыс. Содержание малонового диальдегида в коре больших полушарий увеличивалось через 2 ч. и 4 ч. после окклюзии, далее постепенно снижалось до исходных значений. Активность G-per была снижена по сравнению с контрольными значениями через 30 мин. и 4 ч. после моделирования ишемии. Содержание Nrf2 в коре больших полушарий возрастало через 2 ч. и 4 ч. после проведения окклюзии, при этом через сутки его уровень несколько снижался, а на третий день эксперимента достигал исходных показателей. Количество HIF-1α повышалось только через 24 ч. и 72 ч. после операции.

Заключение. Количество ABCB1-белка в коре головного мозга крыс при глобальной церебральной ишемии зависит от показателей выраженности окислительного стресса, при этом в его регуляции играют роль транскрипционные факторы Nrf2 и HIF-1α. Снижение количества транспортера в гематоэнцефалическом барьере за счет влияния на процессы перекисного окисления липидов или синтез изученных транскрипционных факторов расширяет возможности для повышения эффективности фармакотерапии заболеваний центральной нервной системы субстратами ABCB1-белка.

Ключевые слова: *ABCB1-белок; глобальная церебральная ишемия; перекисное окисление липидов; Nrf2; HIF-1α*

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Regulation of ABCB1 Protein Function in the Cerebral Cortex with the Underlying Global Cerebral Ischemia

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ABSTRACT

INTRODUCTION: ABCB1 is a membrane transporter protein responsible for efflux of a wide range of drugs from cells. The study of the mechanisms of regulation of the functioning of ABCB1 protein in the brain in its ischemia will permit to propose new approaches to pharmacotherapy of cerebral ischemic pathology.

AIM: To study the regulation of ABCB1 protein function in the cerebral cortex of rats with global cerebral ischemia.

MATERIALS AND METHODS: The experiment was performed on 30 male rats with global cerebral ischemia modeled by bilateral occlusion of the common carotid arteries. The amount of ABCB1 protein and Nrf2 and HIF-1α transcription factors in the cerebral cortex was determined by enzyme immunoassay. The free radical status of the cerebral cortex was assessed by the concentration of malondialdehyde, SH groups, and by glutathione peroxidase (G-per) activity.

RESULTS: Bilateral occlusion of the common carotid arteries caused an increase in the level of ABCB1 protein in the cerebral cortex of rats by the 4th hour of ischemia; in 24 hours it remained elevated, and in 72 hours decreased to values that did not differ from those of falsely operated rats. The content of malondialdehyde in the cerebral cortex increased in 2 and 4 hours after occlusion and then gradually decreased to the initial values. In 30 minutes and 4 hours after ischemia modeling, G-per activity decreased compared to the control values. The content of Nrf2 in the cerebral cortex increased in 2 and 4 hours after occlusion, then slightly decreased on the next day, and reached the initial values on the 3rd day of the experiment. The amount of HIF-1α increased only in 24 and 72 hours after the surgery.

CONCLUSION: The amount of ABCB1 protein in the cerebral cortex of rats with global cerebral ischemia depends on the severity of oxidative stress, with Nrf2 and HIF-1α transcription factors playing a role in its regulation. Reduction of the amount of the transporter in the blood-brain barrier through the influence on the lipid peroxidation processes or synthesis of the studied transcription factors expands the possibilities of using ABCB1 protein substrates for improving the effectiveness of pharmacotherapy of diseases of the central nervous system.

Keywords: *ABCB1 protein; global cerebral ischemia; lipid peroxidation; Nrf2; HIF-1α*

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ECO•VECTOR

LIST OF ABBREVIATIONS

G-per — glutathione peroxidase
HIF-1α — hypoxia inducible factor 1α
$NADPH_2$ — nicotine amide adenine dinucleotide phosphate reduced
Nrf2 — nuclear factor erythroid 2-related factor 2 (redox-sensitive transcription factor 2)

INTRODUCTION

ABCB1 protein (*glycoprotein-P*, Pgp) is a membrane transporter protein responsible for efflux of a wide range of lipophilic substrates from cells including medical drugs. Localized in the small intestinal mucosa, on the biliary membrane of hepatocytes, in the epithelium of nephron tubules and in the histo-hematic barriers, the transporter prevents absorption of substrates in the gastrointestinal tract, excretes them into urine and bile and prevents penetration into the sequestrated organs (brain, ovaries, internal eye media, to the fetus).

The functioning of ABCB1 protein is determined by genetic peculiarities of an organism, the existence of pathological conditions, intake of some foodstuffs and medical drugs. Alteration of the activity of the transporter can lead to drug interactions which may result in reduction of the efficiency of pharmacotherapy in case of increase in the activity of the transporter protein, and to increased risk of the relative drug overdose in case of its inhibition [1, 2].

Alteration of the functional activity of ABCB1 protein in the hematoencephalic barrier is a cause for development of multidrug resistance of brain tumors, the ineffectiveness of using neuroprotectors in cerebrovascular disorders, and of epilepsy reluctant to drug treatment [3]. To note, the targeted pharmacological control of ACBC1 protein can increase the effectiveness of pharmacotherapy of these pathologies through increased penetration of transporter substrates into the brain tissue.

The ischemic-hypoxic lesion of the brain is the leading pathogenetic factor in the development of a number of diseases and pathological conditions: strokes, ischemic-hypoxic encephalopathy, discirculatory encephalopathy, metabolic and toxic lesions of the central nervous system.

Most *in vitro* studies of ABCB1 protein have shown increase in its functional activity and synthesis, as well as in the expression of the *MDR1* gene encoding the transporter, on exposure to hypoxic hypoxia [4]. To this end, the functioning of ABCB1 protein in circulatory hypoxia has not been sufficiently studied, and *in vivo* results of the influence of cerebral ischemia and of ischemia/reperfusion on the transporter are rather contradictory.

To note, in the scientific literature, the control of functioning of ABCB1 protein was mostly evaluated *in vitro* on cell cultures over expressing this transporter protein. The results obtained on animals, are scarce and contradictory. A combined participation of redox-sensitive transcription factor 2 (nuclear factor erythroid 2-related factor 2, Nrf2) and of hypoxia inducible factor 1α (HIF-1α) were not considered earlier in the control of the function of ABCB1 protein.

Taking into consideration the above, the identification of the mechanisms controlling ABCB1 protein in the underlying ischemic/hypoxic brain lesions will permit to expand the approach to the targeted alteration of its activity which may increase the effectiveness of pharmacotherapy of the mentioned pathologies through use of these transporter substrates.

The **aim** of this study is regulation of ABCB1 protein functioning in the cerebral cortex of rats with the global cerebral ischemia.

MATERIALS AND METHODS

The experiment was performed on 30 sexually mature male stock Wistar rats with 250 g–360 g body mass, obtained from Stolbovaya nursery (Moscow region, Russian Federation) in accordance with the rules of laboratory practice regulated by the Annex to the Order of the Ministry of Health and Social Development of the Russian Federation of August 23, 2010 No. 708n, and by the Order of the Ministry of Health of the Russian Federation of April 1, 2016 No. 199n 'On the Approval of the Rules of Good Laboratory Practice'. The study was approved by the Commission for control over the maintenance and use of laboratory animals of Ryazan State Medical University (Protocol No. 7 of April 03, 2018). All surgical interventions were performed in the operating room of the Vivarium of Ryazan State Medical University under anesthesia with intraperitoneal administration of Zoletil 50® (Virbac, France) at a dose of 10 mg/kg [3]. The animals were euthanized by taking blood from the

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abdominal aorta under deep anesthesia (intraperitoneal administration of Zoletil 50® at a dose of 30 mg/kg), after which samples of the cerebral cortex were extracted [3].

Experimental series. Rats were divided into 6 series:

- *1 series* — 'false operation' (control),
- *2–6 series* — animals after 30 minute, 2 hours, 4 hours, 24 hours and 72 hours after modeling of global cerebral ischemia by bilateral occlusion of the common carotid arteries of rats.

This operation included dissection of the soft tissues of the neck of an animal, isolation of the common carotid arteries, ligation of them with Surgipro II 4.0 (Covidien, Switzerland) and suturing of the wound.

Preparation of the biological material. The frontal lobe of the cerebral cortex was taken from each animal, the samples were crushed with scissors and homogenized at low temperature in a phosphate buffer with pH 7.4 (1:10 by weight) followed by triple freezing-defrosting for cell lysis, as indicated in the instructions for enzyme immunoassay kit, and centrifugation at 1750 g. The frozen supernatant was stored before analysis at -80° for no more than three weeks.

Determination of the amount of ABCB1 protein in the cerebral cortex. The absolute amount of ABCB1 protein in the cortex was evaluated by the method of heterogeneous enzyme-linked immunoassay with a ready-to-use diagnostic kit Elisa Kits Blue Gene (China) designed for work with rat tissues.

Investigation of the free radical status in the homogenate of the cerebral cortex of rats. *Determination of the concentration of malondialdehyde (thiobarbituric acid — reactive products).* A TBK-AGAT (AGAT-MED, Russia) kit was used to analyze the content of malondialdehyde. The method is based on the ability of malondialdehyde to react with 2-thiobarbituric acid to form an equimolar amount of a colored complex with absorption maxima at 535 nm and 570 nm. Into a tube, 0.25 ml of the supernatant, 3.0 ml of 1.4% orthophosphoric acid, 1.0 ml of 2-thiobarbituric acid solution (150 mg — 31 ml of distilled water) were introduced, after which the tubes were incubated for 45 minutes in water bath at 100°C and cooled with cold water for 3 minutes. After that, 4.0 ml of n-butanol were added, the tubes were shaken until the formation of a homogeneous white suspension with a pink tinge, centrifuged at 1750 g for 10 minutes, and in 3 ml of the organic phase, the optical density was determined on a UV-150-02 spectrophotometer (Shimadzu, Japan) at two wavelengths versus a blank sample (0.25 ml of distilled water instead of brain homogenate). The results were expressed in nmol/mg of protein.

Determination of the content of total sulfhydryl (SH) groups. The method used is based on the ability of sulfhydryl groups to restore disulfide 5.5-dithiobis-2-nitrobenzoate dissolved in ethanol (Ellman reagent) to form an equivalent amount of yellow-colored anions of 2-nitro-5-thiobenzoate, which were determined by the increase in light absorption of the solution on a UV-150-02 spectrophotometer (Shimadzu, Japan) at 412 nm wavelength. The supernatant in the quantity of 100 µl was introduced into a test tube, and 100 µl of 0.2 M ethylenediaminetetraacetate solution in Ellman reagent were added. The optical properties on the resulting mixture were determined on a spectrophotometer after 30-minute incubation at 22°C–25°C.

The content of total sulfhydryl groups was calculated using a molar extinction coefficient of 0.0136 $cm^{-1} \times nmol^{-1}$, the results were expressed in µmol/mg of protein.

Determination of glutathione peroxidase (G-per) activity. The spectrophotometric determination of G-per activity is based on the registration of a decline of the optical density of the test sample at 340 nm and 37°C as a result of the oxidation reaction of reduced nicotine amide adenine dinucleotide phosphate ($NADPH_2$), which is a donor of reducing equivalents for the reaction of enzymatic reduction of glutathione, pre-oxidized by tert-butyl hydroperoxide. The higher the G-per activity, the higher the intensity of $NADPH_2$ oxidation, and the more pronounced decline of the intensity of light absorption.

The reaction mixture contained 1.2 ml of 0.05 mM isotonic phosphate buffer with pH 7.4, 0.1 ml of 1 mM ethylenediaminetetraacetate, 0.1 ml of 0.12 mM $NADPH_2$, 0.2 ml of 1.85 mM reduced glutathione solution, 0.5 units (UN) of glutathione reductase, 0.2 ml of 0.2 mM tert-butyl hydroperoxide and 0.1 ml of the test sample. The reaction was initiated by adding tert-butyl hydroperoxide, after which the tube was incubated at 37°C for 3 minutes. The optical density was determined on a Humalaizer 2,000 biochemical analyzer (Germany) at 340 nm. One unit of activity was taken to be the amount of enzyme required for the oxidation of 1 µM of reduced glutathione per minute under the conditions of determination. The activity results were expressed in nmol $NADPH_2$/min x mg of protein.

Determination of the level of transcription factors. To assess the content of Nrf2 and HIF-1α transcription factors, a sample of cerebral cortex was homogenized in a phosphate buffer with pH 7.4

(1:1 by mass) at low temperature with subsequent triple freeze-defrost for cell lysis, as indicated in the instruction for the enzyme-linked immunoassay kit, and centrifuged at 1750 g. The absolute amount of factors was determined by heterogeneous enzyme-linked immunoassay using Elisa Kits Cloud-Clone (China) and Elisa Kits Blue Gene (China) kits, respectively, designed for work with rat tissue.

The activation of these factors was evidenced by an increase in their amount due to their stabilization under conditions of oxygen deficiency (HIF-1α) or accumulation of lipid peroxidation products (Nrf2) [5].

The absolute amount of ABCB1 protein and transcription factors, as well as the intensity of lipid peroxidation in brain tissue were compared to the total amount of protein determined by Bradford method.

Statistical processing of the results was carried out using Microsoft Office XP package (USA) and Statistica 7.0 program (USA). The character of the data distribution was evaluated using Shapiro–Wilk, Kolmogorov–Smirnov and Lilliefors tests. The equality of variances was proved using Levene test. The intergroup differences were determined by Newman–Keuls test. The dependence of the content of ABCB1 protein in the brain tissue on the parameters of free radical status and the level of transcription factors was assessed by Pearson correlation coefficient (r). The sufficient level of statistical significance was considered to be $p < 0.05$.

RESULTS

The bilateral occlusion of the common carotid arteries was found to cause an unreliable decrease in the amount of ABCB1 protein in the cerebral cortex of rats 2 hours after the onset of pathology, after which its level increased and by the 4th hour of ischemia 3.55 times exceeded the parameters of falsely operated animals ($p < 0.05$). In 24 hours after occlusion, the content of the transporter reliably exceeded (by 3.27 times) that of the false operation series ($p < 0.05$), and in 72 hours its amount decreased to values not differing from the level of falsely operated rats ($p > 0.05$).

In examination of the free radical status of the cerebral cortex of the rats, the following results were obtained by us (Table 1).

Table 1. Content of ABCB1 Protein and Parameters of Intensity of Oxidative Process in Cerebral Cortex of Rats in Bilateral Occlusion of Common Carotid Arteries

Parameters	Series of Experiment					
	False Operation	Bilateral Occlusion of Common Carotid Arteries				
		30 minute	2 hours	4 hours	24 hours	72 hours
n	5	5	5	5	5	5
Level of ABCB1 protein, ng/mg	0.22 (0.16; 0.25)	0.14 (0.064; 0.15)	0.38 (0.21; 0.42)	0.78 (0.40; 0.82)*	0.72 (0.62; 2.08)*	0.22 (0.11; 0.22)
Malondialdehyde, nmol/mg of protein	24.69 (11.21; 33.12)	31.36 (29.41; 40.17)*	68.61 (59.12; 82.54)*	45.46 (24.17; 79.72)*	47.22 (18.48; 69.09)	34.31 (31.10; 38.71)
SH-groups, μmol/mg of protein	48.92 (41.41; 64.01)	24.77 (20.04; 43.31)	55.55 (44.96; 72.67)	42.11 (40.07; 53.31)	31.93 (17.28; 68.08)	40.37 (30.38; 41.49)
Activity of glutathione peroxidase, nmol of NAPDH/mg of protein x min	20.48 (18.6; 22.76)	16.1 (12.7; 19.3)*	20.9 (19.2; 27.4)	15.4 (14.3; 18.0)*	17.04 (16.83; 17.14)	20.71 (19.77; 20.98)

Notes: * — statistically significant differences in comparison with parameters of falsely operated animals ($p < 0.05$); results are presented as median, lower and upper quartiles — in distribution of the data different from normal; $NADPH_2$ — nicotine amide adenine nucleotide phosphate

The content of malondialdehyde in the cerebral cortex statistically increased in 2 hours and 4 hours after occlusion by 2.78 ($p < 0.05$) and 1.84 times ($p < 0.05$), respectively, after which it gradually declined to the initial values. Concentration of the total SH-groups did not reliably differ from the parameters of falsely operated rats, but G-per activity decreased in 30 minute by 21.5% ($p < 0.05$) and

in 4 hours by 24.9% ($p < 0.05$) compared to the control values.

Increase in the amount of ABCB1 protein in the cortex of rats with the global cerebral ischemia was found to correlate with the parameters of lipid peroxidation, which is shown by the direct correlation dependence ($r = 0.33$, trend level, $p = 0.066$) between the level of transporter protein in the cerebral cortex and the amount of malondialdehyde the main parameter characterizing intensification of the tissue lipoperoxidation.

To identify the dependence of the amount of ABCB1 protein on the intensity of oxidative stress in global cerebral ischemia and evaluate the mechanisms of the transporter regulation, the absolute amount of Nrf2 and HIF-1α transcription factors in the cerebral cortex of rats was studied.

Evaluation of the dynamics of the level of transcription factors in the cerebral cortex of rats showed that in 2 hours after modeling bilateral occlusion of the common carotid arteries, Nrf2 content increased 3.83 times ($p < 0.05$), and after 4 hours of ischemia — 4.05 times ($p < 0.05$). At the same time, after a day, the level of this factor decreased but was 1.29 times higher than the trend level ($p = 0.076$). On the 3rd day of the experiment, Nrf2 content reached the initial values (Table 2).

It was established that bilateral occlusion of the common carotid arteries led to a 32.12 times increase in the content of HIF-1α at the trend level ($p = 0.083$) in 24 hours after surgery, and to a reliable increase by 17.21 times ($p < 0.05$) in 72 hours after the experimental effect. At the same time, at other time points, the differences with the parameters of the falsely operated rats were unreliable ($p > 0.05$) (Table 2).

Table 2. Content of Nrf2 and HIF-1α Transcription Factors in Cortex of Rats in Bilateral Occlusion of Common Carotid Arteries

Parameters	Series of Experiment					
	False Operation	Bilateral Occlusion of Carotid Arteries				
		30 minute	2 hours	4 hours	24 hours	72 hours
n	5	5	5	5	5	5
Nrf2 amount, ng/mg of protein	0.021 (0.013; 0.031)	0.011 (0.0052; 0.014)	0.038* (0.028; 0.044)	0.085* (0.015; 0.122)	0.027** (0.023; 0.031)	0.003 (0.0021; 0.0062)

Notes: * — cstatistically significant differences in comparison with parameters of falsely operated animals ($p < 0.05$); ** — differences in comparison with parameters of falsely operated rats at the trend level ($0.05 < p < 0.1$); HIF-1α — hypoxia inducible factor 1α, Nrf2 — nuclear factor erythroid 2-related factor 2 (redox-sensitive transcription factor 2)

The correlation analysis identified a direct dependence between the amount of ABCB1 protein and content of Nrf2 in the cortex of rats in bilateral occlusion of the common carotid arteries ($r = 0.33$, $p = 0.047$).

DISCUSSION

To study the functioning of ABCB1 protein in the cerebral cortex of large hemispheres, we have chosen a model of bilateral occlusion of the common carotid arteries of male rats, which is an adequate model of a chronic cerebral hypoperfusion [6].

It was found that in bilateral occlusion of the common carotid arteries, the level of ABCB1 protein in the brain began to rise starting from the 4th hour after the onset of pathology and remained elevated within one day of the experiment. To note, ischemia lasting more than 3 days, has not been studied because of the low survival rate of the animals, which agrees with the literature data [7], and also because of partial restoration of the cerebral circulation due to neoangiogenesis in the survived rats [8], which would lead to incorrect interpretation of the data.

In the scientific literature, a direct correlation dependence between the amount of ABCB1 protein and its functional efflux activity has been demonstrated [9]. Accordingly, an increase in the amount of ABCB1 protein in the cerebral cortex in global cerebral ischemia will lead to enhancement of its functioning, which will be manifested by a decrease in the penetration of protein-transporter substrates, including drugs, into the brain tissue.

The results of such studies in the scientific literature are scarce and contradictory. Thus, it was found that a 20-minute occlusion of the vertebral and

carotid arteries caused an increase in the concentration of doxorubicin, a substrate of ABCB1 protein, in the brain of rats, which, according to the authors, is associated with depletion of adenosine triphosphate in the brain tissue and a decrease in the activity of the transporter [10]. The differences between our results and the data from other experiments may be due to the different amount of cerebral ischemia, the duration and method of modeling pathology, the severity of oxidative stress, the area of tissue sampling, and different methods of analysis.

Activation of lipid peroxidation is a sign of a number of diseases of the central nervous system, including those of ischemic etiology [11]. When studying the free radical status of the rat cerebral cortex, it was found by us that an increase in the amount of ABCB1 protein in the cerebral cortex in global cerebral ischemia correlates with the parameters of lipid peroxidation, which is evidenced by the revealed direct correlation between the level of the transporter in the brain and the amount of malondialdehyde the main parameter characterizing the intensification of lipid peroxidation. The obtained dependence is consistent with the results of other scientific papers, where ischemia was found to be associated with activation of the expression and functional activity of ABCB1 protein, correlating with the intensification of lipid peroxidation.

Thus, it was shown in *in vitro* experiment that the effect of hydrogen peroxide (200 μM) led to oxidative stress and to an increase in the amount of ABCB1 protein and *MDR1a* and *MDR1b* transcripts in the culture of endothelial cells of the cerebral vessels of rats [12].

Incubation of KB31, KBV1, A549 and DMS-53 tumor cell cultures on hypo- and hyperglycemic media induced development of oxidative stress via NADPH2-dependent oxidase 4 and destabilization of mitochondrial membranes, increase in expression and activation of HIF-1 transcription factor and evident increase in the functional activity of ABCB1 protein leading to resistance of its substrate — doxorubicin [13].

It should be noted that the scientific literature also presents the data on the inhibition of ABCB1 protein in activation of lipid peroxidation.

Thus, in ischemia/reperfusion of the intestine, reduction of excretion of an ABCB1 protein substrate — rhodamine-123 by the liver was noted, which may be due to decreased expression of transporter in result of increased peroxide oxidation of lipids and increased content of information ribonucleic acid of inducible NO-synthase [14].

In *in vitro* experiment it was established that incubation of cells over expressing ABCB1 protein with low doses of hydrogen peroxide (0.1 μM–1.0 μM), resulted in increase in the amount of the transporter, with no changes in its activity. When the concentration of the pro-oxidant increased to 10 μM, the functional activity of the transporter decreased, and at concentrations 50 μM–100 μM, there was a reduction of its quantity and activity [15].

Inhibition of ABCB1 protein against the background activation of peroxide oxidation of lipids in the above experiments is probably associated with the development of a more prominent uncompensated oxidative stress.

To identify the dependence of the amount of ABCB1 protein of the intensity of oxidative stress in global cerebral ischemia and to evaluate the mechanisms of regulation of the transporter, the absolute amount of Nrf2 and HIF-1α transcription factors in the cerebral cortex of rats was studied. Nrf2 is an intracellular sensor of reactive oxygen species and pro-oxidants and an activator of a number of detoxifying enzymes and efflux transporters of xenobiotics and toxic metabolites including ABCB1 [16], and HIF-1 is a sensor of tissue hypoxia [17].

Evaluation of the dynamics of the level of transcription factors in the cerebral cortex of rats showed that the content of Nrf2 increased in 2 hours, 4 hours and 24 hours after bilateral occlusion of the common carotid arteries. On the 3rd day of the experiment, Nrf2 content returned to the initial values. The amount of HIF-1α increased only in 24 hours and 72 hours after surgery. At the same time, a correlation analysis revealed a direct dependence between the amount of ABCB1 protein and the content of Nrf2 in the cerebral cortex, which demonstrates the role of this factor in regulating the level of the transporter protein. This is confirmed in *in vitro* experiments, which show prevention of increase in the amount of ABCB1 protein in hypoxia in result of knockdown of the gene encoding Nrf2 [18].

In another *in vitro* study, inhibition of HIF-1 transcription factor was found to prevent Increase in the content of ABCB1 protein in hypoxia [19]. To note, the absence of any reliable changes in HIF-1α within the first hours of ischemia in our experiment is likely to be connected with inter-individual differences between the animals. It is also known that synthesized Nrf2 is rapidly degraded by 26S proteasome in cell cytoplasm, and formation of HIF-1α is characterized by a long latent period and a longer functioning due to persisting oxygen deficit [20]. Taking into account the results obtained *in vitro* and our results, it can be suggested that at the early stages of ischemia, the main regulator of ABCB1 protein is Nrf2, and at later stages — oxygen-sensitive factor.

CONCLUSION

Thus, the amount of ABCB1 protein in the cerebral cortex of rats in global cerebral ischemia depends

on the severity of oxidative stress. The role in its regulation is played by Nrf2 and HIF-1α transcription factors. Decline in the amount of the transporter in the hematoencephalic barrier through the influence on lipid peroxidation processes or synthesis of the studied transcription factors, expands the possibilities of increasing the penetration of ABCB1 protein into the brain to increase the effectiveness of pharmacotherapy of various diseases of the central nervous system, where the intensification of work of the transporter was demonstrated.

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Contribution of the authors: *I. V. Chernykh* — concept of study, execution of study; *A. V. Shchul'kin* — analysis and processing of data; *N. M. Popova* — analysis and processing of data , writing the text; *M. V. Gatsanoga* — execution of study, methodology development; *E. N. Yakusheva* — research management. The authors confirm the correspondence of their authorship to the ICMJE International Criteria. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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Вклад авторов: *Черных И. В.* — концепция исследования, проведение исследования; *Щулькин А. В.* — анализ и курирование данных; *Попова Н. М.* — анализ и курирование данных, написание текста; *Гацанога М. В.* — проведение исследования, разработка методологии; *Якушева Е. Н.* — руководство научно-исследовательской работой. Все авторы подтверждают соответствие своего авторства международным критериям ICMJE (все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией).

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