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EVALUATION OF ABCB1-PROTEIN INHIBITION PROSPECTIVENESS IN HEMATOENCEPHALIC BARRIER AS METHOD FOR INCREASING EFFICIENCY OF PHARMACOTHERAPY IN CEREBRAL ISCHEMIA

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The aim of the article is to evaluate the ABCB1 protein inhibition in the blood-brain barrier to increase the effectiveness of neuroprotective therapy for focal and global cerebral ischemia.

Materials and methods. The work was performed on 103 male Wistar rats. In the 1st group (n=33), the neuroprotective activity of the ABCB1 protein substrate, nimodipine (0.4 mg/kg), was analyzed in terms of reducing the area of the brain necrosis after a 1-hour occlusion of the middle cerebral artery with a 24-hour reperfusion (focal ischemia). In the 2nd group (n=60), the effectiveness of nimodipine was analyzed by reducing the lethality of rats and the neurological deficit (ND) level against the background of the bilateral occlusion of the common carotid arteries (global ischemia). In both groups, nimodipine was used alone or in the combination with omeprazole, the ABCB1 protein inhibitor (17.6 mg/kg). The drugs were administered intravenously.

Results. The nimodipine administration to the 1st group led to the reduction of the necrosis focus by 28% compared with the control series. Omeprazole did not cause a change in the area of the necrosis. The combination of drugs caused a decrease in the area of the necrosis in relation to the control by 29%; there were no differences in comparison with the nimodipine series. Nimodipine reduced the rats' lethality in the 2nd group against the background of the pathology (a tendency). Omeprazole alone did not change the mortality. The drug combination reduced the mortality compared to the control and nimodipine series. The administration of omeprazole alone did not reduce the neurological deficit score relative to the control. In the nimodipine series, ND was 88% lower than the control, after 24 hours. With the administration of the drugs combination, this indicator decreased in relation to the control by 88%, 80%, 88%, 87% and 86% after 4, 12, 24, 48 and 72 hours, respectively, and in relation to the nimodipine series it decreased by 60% and 67% after 4 and 48 hours.

Conclusion. The ABCB1 protein inhibition is promising for increasing the effectiveness of neuroprotective therapy for global ischemia, but not for focal cerebral ischemia.

Keywords: ABCB1 protein; occlusion-reperfusion of the middle cerebral artery; bilateral occlusion of the common carotid arteries; nimodipine; omeprazole

Abbreviations: BBB – blood-brain barrier; TTC – triphenyltetrazolium chloride; ATP – adenosine triphosphate; creb – cAMP response element-binding; Akt – protein kinase B (intracellular protein kinase B enzyme); IC_{50} – half-maximal (50%) inhibitory concentration.

ОЦЕНКА ПЕРСПЕКТИВНОСТИ ИНГИБИРОВАНИЯ ABCB1-БЕЛКА В ГЕМАТОЭНЦЕФАЛИЧЕСКОМ БАРЬЕРЕ КАК МЕТОДА ПОВЫШЕНИЯ ЭФФЕКТИВНОСТИ ФАРМАКОТЕРАПИИ ЦЕРЕБРАЛЬНОЙ ИШЕМИИ

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Цель. Оценка ингибирования ABCB1-белка в гематоэнцефалическом барьере с целью повышения эффективности нейропротекторной терапии фокальной и глобальной церебральной ишемии.

Материалы и методы. Работа выполнена на 103 крысах-самцах линии Вистар. На 1-й группе (n=33) анализировали нейропротекторную активность субстрата ABCB1-белка – нимодипина (0,4 мг/кг) по снижению площади некроза головного мозга после 1-часовой окклюзии средней мозговой артерии с 24-часовой реперфузией (фокальная ишемия). На 2-й группе (n=60) эффективность нимодипина анализировали по снижению летальности крыс и уровня неврологического дефицита (НД) на фоне билатеральной окклюзии общих сонных артерий (глобальная ишемия). В обеих группах нимодипин использовался отдельно или в сочетании с ингибитором ABCB1-белка – омепразолом (17,6 мг/кг). Препараты вводились внутривенно.

Результаты. Введение нимодипина 1-й группе привело к сокращению очага некроза на 28% по сравнению с серией контроля. Омепразол не вызвал изменения площади некроза. Комбинация препаратов вызвала снижение площади некроза по отношению к контролю на 29%, в сравнении с серией нимодипина различий не было. Нимодипин сокращал летальность крыс 2-й группы на фоне патологии (тенденция). Омепразол не изменял летальность. Комбинация препаратов снижала летальность по сравнению с сериями контроля и нимодипина. Введение омепразола не сокращало балл неврологического дефицита относительно контроля. В серии нимодипина НД был ниже контроля через 24 ч. на 88%. При введении комбинации препаратов данный показатель снижался по отношению к контролю через 4, 12, 24, 48 и 72 ч. на 88%, 80%, 88%, 87% и 86%, а по отношению к серии нимодипина через 4 и 48 ч – на 60% и 67%.

Заключение. Ингибирование ABCB1-белка является перспективным для повышения эффективности нейропротекторной терапии глобальной ишемии, но не фокальной ишемии мозга.

Ключевые слова: ABCB1-белок; окклюзия-реперфузия средней мозговой артерии; билатеральная окклюзия общих сонных артерий; нимодипин; омепразол

Список сокращений: ГЭБ – гематоэнцефалический барьер; ТТХ – трифенилтетразолия хлорид; АТФ – аденозинтрифосфат; CREB – белок, связывающийся с цАМФ-зависимым элементом; АКТ – внутриклеточный фермент протеинкиназы B; IC_{50} – концентрация полумаксимального ингибирования.

INTRODUCTION

P-glycoprotein (ABCB1-protein, Pgp) is a product of the MDR1 gene expression, as well as a membrane efflux ATP-dependent transporter protein with a wide range of substrates different in their chemical structure. In significant amounts, it is localized on the biliary surface of hepatocytes, in the proximal tubules of the renal nephrons, in the enterocytes of the small intestine and in the endotheliocytes of the histohematic barriers. One of the leading functions of the ABCB1 protein in the blood-brain barrier (BBB) is to prevent the penetration of lipophilic endogenous and exogenous substrate substances from the blood into the brain. It should be notified that the functional activity of the ABCB1 protein can vary significantly against the background of various influences, i.e., the inflammatory process, oxygen deficiency, oxidative stress, food intake, and a lot of drugs [1].

By far, a stroke is the leading cause of disability in the adult population and the second leading cause of death in the world. In 85% of cases, an ischemic type of stroke occurs. At the same time, the use of a tissue plasminogen activator, which is one of the few approved drugs for restoring a blood flow, is limited by a narrow “therapeutic window” (4.5 hours). Thus, the preferred therapeutic strategy for a stroke is neuroprotection, e. i. maintaining the viability of neurons in the penumbra zone [2].

Among the ABCB1 protein substrates, there is a number of drugs with a proven or potential neuroprotective activity: nimodipine, acetylcholinesterase inhibitors rivastigmine, donepezil, galanthamine, the antipsychotic drug paliperidone, and a number of others [3–5]. The substances inefficiency of this pharmacological group in clinical trials, demonstrated by foreign researchers [6], may be due to their insufficient transport through the BBB [7], for example, due to ABCB1-protein efflux. The indirect evidence of such a theory is the activity induction and expression of the transporter against the background of oxygen deficiency, the main pathogenetic link in cerebral ischemia, which was revealed in *in vitro* and *in vivo* experiments [8, 9]. Due to the wide spread of ischemic stroke and chronic cerebral ischemia in the world and, in particular, in Russia [10], the study of such pathogenetic mechanisms and an attempt to overcome them is beyond doubt.

The analysis of the available scientific data suggested the prospect of pharmacological inhibition of the ABCB1 protein in the BBB against the background of cerebral ischemia in order to intensify the delivery of neuroprotectors to the brain and enhance their central effects. The selectivity of reducing the local ABCB1 protein activity in the barrier will minimize the number of pharmacokinetic and pharmacodynamic complications associated with the important functions of the transporter in controlling not only penetration into the brain, but also the enteral absorption and excretion of the drug substrates [1].

THE AIM of the article is to evaluate the ABCB1 protein inhibition in the blood-brain barrier, to increase the effectiveness of pharmacotherapy for the consequences of focal and global kinds of cerebral ischemia in the *in vivo* experiment.

MATERIALS AND METHODS

Laboratory animals

The work was performed on 103 male Wistar rats weighing 200–280 g. The animal manipulations were carried out in accordance with the rules of good laboratory practice (Order of the Ministry of Health of the Russian Federation dated April 1, 2016, No. 199n, and also with international standards (Guide for the Care and Use of Laboratory Animals: VIII ed.) under the operating conditions of a conventional vivarium, and approved by the Commission for the Control of the Maintenance and Use of Laboratory Animals (Protocol No. 7 dated April 3, 2018) of Ryazan State Medical University. The surgical interventions were carried out against the background of the intraperitoneal administration of Zoletil® 50 (INN – tiletamine, zolazepam; Virbac, France) to the rats at the dose of 10 mg/kg. The intravenous administration of the drugs was carried out in the rats' tail vein.

Experiment design

The experimental animals were divided into 2 groups.

In the first group (n=33), the possibility of the ABCB1 protein pharmacological inhibition was analyzed in order to increase the treatment effectiveness of the focal cerebral ischemia transporter with neuroprotective drugs – substrates. That kind of ischemia was an experimental analogue of an ischemic stroke in humans [11]. A neuroprotective activity was assessed by a decrease in the area of the necrosis focus against the background of the brain ischemia-reperfusion. It had been modeled by a 60-minute endovascular occlusion of the middle cerebral artery with a polypropylene thread with a diameter of 4–0 (0.15–0.199 mm) together with a permanent ligation of the common and external carotid arteries followed by recanalization. During the surgical procedures and for 2 hours after them, the animals were warmed up with lamps to maintain the rectal temperature at 37°C. The animals were withdrawn from the experiment 24 hours after the reperfusion by the overdose of Zoletil (30 mg/kg). A similar model is widely used in scientific studies [12, 13]. The rats with a lethal outcome were not included in the study and not reflected in the total number.

The animals of the first group were divided into 4 series, each of 7 animals: Series 1 – the animals with ischemia-reperfusion with a saline intravenous administration (1 ml/kg) at the time of the middle cerebral artery reperfusion. The rats of the 2nd series were subjected to the ischemia-reperfusion with an intravenous injection of the ABCB1 protein substrate, a cerebral vasodilator with a neuroprotective activity, nimodipine (Nimotop, Russia), at the dose of 0.4 mg/kg of the animal body weight (the solution 0.4 mg/ml − 1 ml/kg) [14] at the time of reperfusion. The neuroprotective activity of nimodipine, regardless of the cell type, had been shown in numerous studies both *in vitro* and *in vivo*. According to the latest data, the main role is assigned to the prevention of a stress-induced apoptosis by reducing the activity of caspase-3 and 7, as well as by activating the transcription factor, the protein that binds to the cAMP-responsible element (creb) and the intracellular enzyme protein kinase B (Akt) of signaling pathways [15]. The animals of the 3rd experimental series were the rats with ischemia-reperfusion with an intravenous injection of the ABCB1 protein inhibitor in the BBB, omeprazole (Omez, Russia) at the dose of 17.6 mg/kg of body weight (the solution 17.6 mg/ml – 1 ml/kg) [16, 17] at the time of reperfusion. The 4th series were the animals with ischemia-reperfusion with the introduction of nimodipine in combination with an intravenous injection of omeprazole at the time of recanalization (the drugs were administered sequentially without any interruption).

The dose and scheme of the neuroprotective substrate ABCB1 protein (nimodipine) administration used in this work, is explained by the revealed efficiency in reducing the volume of necrosis in the experiment [14]. Typical neuroprotective drugs (piracetam, etc.) were not used in the work due to the absence of the drugs – substrates of the ABCB1 protein – among them.

The proton pump blocker omeprazole was chosen as a transporter inhibitor. The drugs of this pharmacological group, such as omeprazole, pantoprazole and lansoprazole, demonstrated an inhibitory activity against the ABCB1 protein on Caco-2 and L-MDRI cell cultures with a half-maximal inhibition concentration (IC_{50}) of 17.7, 17.9 and 62.8, respectively. μM, which was revealed by the degree of translocation of the transporter substrate, digoxin [17]. When omeprazole is administered to rats intravenously at the dose of 3.45 mg/kg, its maximum plasma concentration is about 3.5 μM [16]. To obtain 17.7 μM (IC_{50} in relation to the ABCB1 protein for this substance), it must be administered by 5.1 times more, i.e. 17.6 mg/kg. This is confirmed by the linearity of the pharmacokinetics of omeprazole with its single administration [18].

Despite the fact that omeprazole slightly reduces the activity of microsomal liver enzymes *in vitro* [19], its administration to the rats at the indicated dose cannot affect the intensity of nimodipine biotransformation, due to the fact that only the CYP3A isoform is involved in the metabolism of the latter [20].

In addition, 5 animals underwent "a sham operation" with opening the skin and soft tissues of the neck without any direct occlusion-reperfusion of the arteries to confirm the absence of the influence of experimental manipulations on the results of the study.

To assess the brain necrosis zone in the animals of all the series, the brain was removed, washed in an isotonic sodium chloride solution, followed by freezing and cutting in the frontal plane into sections 2 mm thick. Next, the sections were placed in a Petri dish, 10 ml of phosphate buffer with pH=7.4 was added to them, then

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2 ml of a 2% solution of triphenyltetrazolium chloride (TTC) was added and then 2 ml of a 2% solution of sodium succinate was added. The sections were kept in the indicated mixture in the thermostat at 38°C for 1 h, then at room temperature in 10% neutral formalin for also 1 h. Subsequently, the sections were photographed with a Canon Power Shot G5 digital camera. The percentage ratio of the sum of the TTX-negative zones areas to the sum of the total brain area was estimated [21].

Additionally, to explain the results obtained in the first group of the animals, the integrity of the BBB was studied against the background of the occlusion-reperfusion of the middle cerebral artery. Herewith, the degree of the Evans blue dye accumulation (Evans blue, Sigma, USA) in the brain tissue by the intravenous administration of a 2% isotonic solution in the volume dose of 0.4 ml at 100 g in the tail vein at the time of reperfusion was analyzed. Under the deep anesthesia, immediately before decapitation, the rats underwent transcardial perfusion with 100 ml of an isotonic solution to remove the intravascular dye. Then the animals were withdrawn from the experiment with the extraction of the cerebral cortex (the side supplied by the occluded artery) and its grinding with scissors. The dye was extracted by homogenizing the tissue in dimethylformamide (1 ml at 100 mg of the tissue) at 26,000 rpm for 1 min and followed by incubating at 60°C for a day in a thermostat. The resulting suspension was centrifuged for 5 min at 1750 g.

The Evans blue concentration was determined spectrophotometrically (Bio-Rad spectrophotometer, USA) at 620 nm. The quantitative determination was carried out by the method of an external standard in a similar solvent [22]. The experiment was repeated on 5 rats, 5 rats were used as control, which reproduced "the sham operation".

In the second group of rats (n=60), the prospects of the ABCB1 protein pharmacological inhibition were evaluated in order to increase the effectiveness of treatment with neuroprotective drugs that are substrates of the global cerebral ischemia transporter, an experimental pathology similar in pathogenesis to cerebral hypoperfusion in humans [23]. The neuroprotective potential was analyzed by the decrease in the percentage of the animals' deaths and the severity of a neurological deficit according to the McGrow Stroke-index scale modified by I.V. Gannushkina against the background of a bilateral ligation of the common carotid arteries lasting 4, 12, 24, 48, and 72 hours. In each series, a number of rats with mild (0.5–2.5 points), moderate (2.5–5.5 points), and severe (5.5–10 points) neurological deficits was assessed. This scale is recommended for assessing the neurological status of animals in preclinical studies[1].

The animals of the second group were divided into 5 series: the 1st (n=6) were sham-operated animals (a control series), the 2nd (n=14) were the rats that were simulated cerebral ischemia by the bilateral occlusion of the common carotid arteries intravenously administrated with physiological saline (1 ml/kg) 30 minutes before pathology (the pathology control). Group 3 (n=13) were the animals that were intravenously injected with the neuroprotector nimodipine, a substrate of the ABCB1 protein, at the dose of 0.4 mg/kg 30 min before the arterial ligation. The 4th group (n=11) were the rats that were intravenously injected with a transporter inhibitor omeprazole at the dose of 17.6 mg/kg body weight 30 min before the pathology. The 5th group (n=16) were the animals with cerebral ischemia, intravenously injected with a combination of nimodipine and omeprazole in the similar doses 30 min before pathology modeling.

Statistical processing of results

The results of the study were processed using the Statistica 13.0 program. The nature of the data distribution was assessed by the Shapiro-Wilk test. To compare the size of the necrosis zone, the ANOVA analysis of variance and the Newman-Keuls test for a pairwise comparison were used. The comparison of the animals' neurological deficit level was performed using the Kruskal-Wallis test (an analogue of the analysis of variance for the data distributed in a non-normal way), pairwise comparisons – the Mann-Whitney test with the Bonferroni correction, which allows analyzing samples of different sizes. The animals' survival in the postoperative period was assessed by constructing Kaplan-Meier survival curves. The survival comparison was assessed by the Cox F-test. The differences were considered significant at the confidence level more than 95%.

RESULTS

In the first group, the lethality of the animals after modeling the occlusion-reperfusion of the middle cerebral artery was 22.7±1.1% and did not differ in the groups ($p>0.05$). There was no mortality in the group of "sham-operated" animals.

Fig. 1 shows the brain sections of the experimental animals' samples in various series.

In all the experimental series, in percent, the sizes of the necrosis foci are shown in Table 1 (arithmetic mean ± standard deviation).

A 60-minute occlusion of the middle cerebral artery followed by recanalization for 24 hours, led to the formation of a necrosis focus in the control rats' brain with a relative area of 32.2±7.1%. The similar results correspond to the literature data [24].

An isolated administration of the L-type calcium channel blocker neuroprotector nimodipine to the animals at the time of the middle cerebral artery reperfusion led to a significant decrease in the size of the necrotic lesion by 27.7% compared with the pathology control group ($p<0.05$).

[1] Mironov AN. Rukovodstvo po provedeniyu doklinicheskih issledovanij lekarstvennyh sredstv [Guidelines for conducting preclinical studies of drugs]. Part 1. Moscow: Grif and K. 2012. – 944 p. Russian

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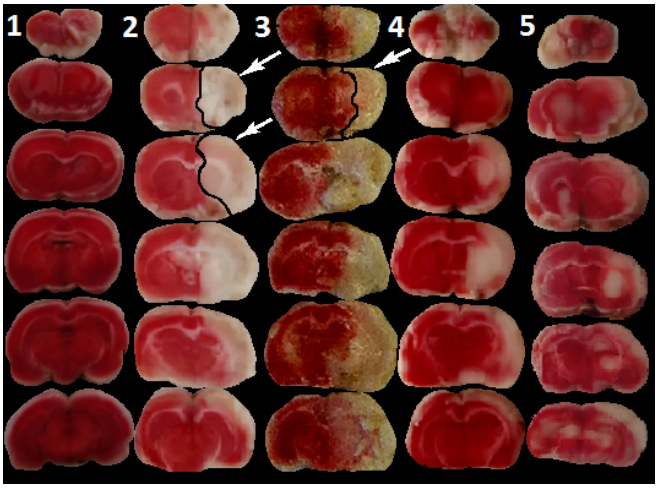

Figure 1 – Samples of rats' brain sections after the occlusion-reperfusion of the middle cerebral artery
Note: 1 – sham operation; 2 – pathology control; 3 – omeprazole; 4 – nimodipine; 5 – combination of nimodipine and omeprazole; arrows indicate the area of brain necrosis.

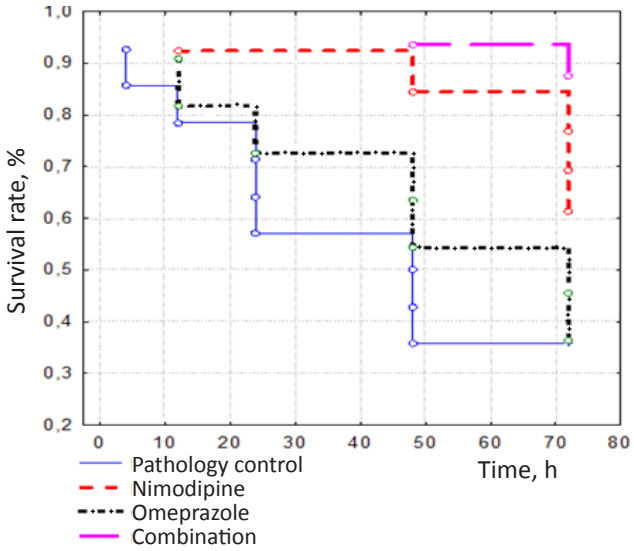

Figure 2 – Graph of cumulative proportion of survived animals according to Kaplan-Meier in different experimental series

Table 1 – Relative area of the rats' brain necrosis zone against the background of occlusion-reperfusion of the middle cerebral artery

Experiment series	Relative area of necrosis, %
Sham operation	0
Control	32.2±7.1
Omeprazole	34.4±7.6
Nimodipine	23.3±4.7*#
Nimodipine + omeprazole	23.0±2.8*#

Note: * – significant differences with the parameter of the pathology control series; # – significant differences with the omeprazole series.

Table 2 – The content of Evans blue dye in the rats' brain against the background of occlusion-reperfusion of the middle cerebral artery

Experiment Series	Dye level, μg/g brain
Sham operation, n=5	2.39±0.85
Occlusion-reperfusion of the middle cerebral artery, n=5	11.55±8.40

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Table 3 – The degree of neurological deficit according to McGrow in the modification by I.V. Gannushkina against the background of bilateral occlusion of the common carotid arteries

Series	Degree of neurological deficit, points				
	4 hr	12 hr	24 hr	48 hr	72 hr
Sham operation,	0.5±0.11	0	0	0	0
Pathology control	4.5 (2.0; 5.5)	5.0 (2.0; 10.0)	8.5 (1.5; 10.0)	7.5 (1.5; 10.0)	7.0 (2.0; 10.0)
Nimodipine	2.5 (2.5; 2.5)	3.0 (1.0; 4.0)	1.0 (1.0; 2.5)*#	3.0 (2.0; 3.0)#	1.5 (1.0; 2.0)#
Omeprazole	2.5 (2.5; 3.5)	3.5 (2.0; 5.0)	3.5 (3.0; 10.0)"	10.0 (3.5; 10.0)"	10.0 (4.5; 10.0)"
Combination	1.0 (0.0; 1.0)*#"	2.0 (1.0; 5.0)*	1.0 (1.0; 3.0)*#	1.0 (0.0; 3.0)*#"	1.0 (1.0; 3.0)*#

Note: * – significant differences with the pathology control group (p<0.05); # – significant differences with the omeprazole group (p<0.05); " – significant differences with the nimodipine group (p<0.05). Data are presented as median, lower and upper quartiles.

An isolated administration of the ABCB1 protein inhibitor omeprazole to the animals did not cause a statistically significant change in the area of the necrosis focus (p>0.05).

A combined administration of nimodipine and omeprazole to the rats caused a decrease in the area of necrosis compared to the pathology control by 28.8% (p<0.05), and in relation to the omeprazole group – by 33.2% (p<0.05). However, no statistically significant changes between the groups of the drugs combination and an isolated administration of nimodipine have been revealed (p>0.05).

The occlusion-reperfusion of the rats' middle cerebral artery led to the impaired BBB permeability, which was confirmed by the accumulation of Evans blue diazo dye in the ischemic brain tissue of the animals. Normally, it does not penetrate into the brain due to the strong bonds with blood plasma albumins [22]. Against the background of the ischemia-reperfusion, the level of the dye in the brain exceeded that of the sham-operated animals by 4.83 times (p<0.05) (Table 2).

The appearance of the dye in the non-ischemic brain of sham-operated rats is probably due to its adsorption on the vessel wall after the perfusion procedure.

Fig. 2 shows a graph of the survived animals' cumulative proportion in the second group according to Kaplan-Meier. None of the sham-operated animals died during the entire observation period. The animals' deaths in the pathology control group was observed 4 hours after the operation; by the end of the first day, 57.1% of the rats died.

The injection of nimodipine before cerebral ischemia led to a decrease in the number of dead animals compared with the pathology control series at the tendency level (0.05<p<0.1). The administration of omeprazole before ischemia did not cause significant changes in the lethality of the animals compared with ischemia control. The combination of nimodipine and omeprazole resulted in the lower mortality in the both pathology control series and in the nimodipine administration series (p<0.05).

The severity and manifestations of ischemia were clinically analyzed by assessing neurological abnormalities according to the McGrow scale modified by I.V. Gannushkina (Table 3). The sham-operated animals showed no signs of neurological deficit (with the exception of minor changes after 4 hours, probably associated with the recovery from anesthesia). After the bilateral occlusion of the common carotid arteries, the survived of animals showed such symptoms as lethargy, unilateral and bilateral blepharoptosis, general tremor, circling behavior, paresis of the extremities. The score of neurological deficit in the pathology control group increased until the end of the first day after the surgery, then decreased, but at all the periods, except 4 hours, it was classified as severe.

Thus, the permanent occlusion of the common carotid arteries of the rats was accompanied by a high mortality of the animals and the development of a severe neuropsychiatric deficit.

The omeprazole injection to the rats 30 min before pathology did not lead to a significant reduction in the neurological deficit score compared to the pathology control at any of the observed periods (p>0.05). In the animals treated with nimodipine, the score of neurological deficit after the surgery was lower than in the control animals: after 24 hours – by 88.2% (p<0.05); after 4 and 72 hours – by 44.4% and 16.0%, respectively, at the trend level (0.05<p<0.1). The administration of nimodipine also led to a decrease in the level of neurological deficit compared to the omeprazole group: after 24 hours – by 71.4% (p<0.05), after 48 hours – by 70.0% (p<0.05) and after 72 hours – by 85.0% (p<0.05).

The administration of a substrate and the ABCB1 protein inhibitor combination to the rats resulted in a decrease in the neurological deficit score compared to all the experimental series. In relation to the pathology control, the level of neurological deficit was lower after 4, 12, 24, 48 and 72 hours by 87.5% (p<0.05), 80.0% (at the level of a pronounced trend, p=0.05), 88.2% (p<0.05), 86.7% (p<0.05) and 85.7% (p<0.05), respectively. In comparison with the group of the omeprazole administration, after 4, 24, 48 and 72 hours it was lower by 60.0% (p<0.05), 71.4% (p<0.05), 90.0% (p<0 .05) and 90.5% (p<0.05), respectively; and compared with the nimodipine series, after 4 hours it was lower by 60.0% (p<0.05), after 48 hours – by 66.7% (p<0.05).

DISCUSSION

The study analyzed the feasibility of the local pharmacological inhibition of the ABCB1 protein in the BBB as a way to increase the effectiveness of neuroprotectors in cerebral ischemia. Moreover, two pathological models were chosen: global ischemia, which causes damage to the white matter of the brain, similar to that in chronic cerebral hypoperfusion in humans [23], and focal ischemia, an experimental analogue of an ischemic stroke [11]. Rats were chosen as a test system for analyzing the ABCB1 protein functioning against the background of cerebral ischemia, due to the demonstrated 93% similarity of the substrates and the modulators spectrum of the transporter activity in these animals and humans [25].

To date, the attempts to reduce the activity of the ABCB1 protein transporter to intensify the delivery of cytotoxic agents to tumor cells and overcome the phenomenon of multidrug resistance (in the formation of which the hyperfunction of the ABCB1 protein plays an important role) have not been successful due to a significant number of pharmacokinetic and pharmacodynamic limitations. On the other hand, the strategy of the ABCB1 protein inhibition in the BBB in the experiments to increase the delivery of drugs, in particular neuroprotective drugs, to the brain, is being successfully developed. Thus, in nonhuman primates, the efficiency of reducing the activity of the transporter in the BBB by infusion of the specific inhibitor elacridar in order to increase the cerebral penetration of the ABCB1 protein substrate, erlotinib was found [26]. It was also established by positron emission tomography that the permeability of the baboon barrier for the labeled radioactive substrate ABCB1-protein [(11)C]-N-desmethyl-loperamide against the background of an intravenous administration of a therapeutic dose (15 mg/kg/h) of cyclosporine (a transporter inhibitor), was significantly increased [27].

The results of the carried out work indicate that a local decrease in the activity of the ABCB1 protein in the BBB during focal cerebral ischemia is not justified. A decrease in the activity of the transporter at the system level, which is more easily achievable in practice, is dangerous due to the changes in the pharmacokinetics of its substrates and the possibility of their relative overdose. A probable reason for the obtained results is an increase in the permeability of the BBB against the background of the occlusion-reperfusion of the middle cerebral artery, which is a typical consequence of cerebral ischemia. In addition, in a previous work, the authors even found a decrease in the functional activity of the ABCB1 protein in the BBB when modeling the occlusion-reperfusion of the middle cerebral artery. That had been revealed by the accumulation degree of the transporter marker substrate, fexofenadine, in the brain [28], despite an increase in the amount of ABCB1-protein against the background of focal cerebral ischemia. In this regard, the inefficiency of a combined use of the neuroprotective substrate ABCB1 protein with the inhibitor of the transporter functional activity, is probably a consequence of a large area of the brain damage in which the structure of the BBB is disturbed, and its permeability is already maximum. This assumption was confirmed by the authors' analysis of the Evans blue dye accumulation in the brain against the background of the applied experimental pathology. At the same time, the ABCB1 protein, most likely, is no longer an obstacle to the penetration of nimodipine into the brain tissue, so the drug has the same maximum therapeutic effect as when administered in isolation.

Other dynamics was obtained by the authors using the model of global cerebral ischemia. In this kind of ischemia, such a pronounced violation of the BBB integrity probably does not occur. The ABCB1 protein pharmacological inhibition in the barrier led to a more significant cerebroprotective effect of nimodipine, manifested by a decrease in mortality and the severity of neurological deficit in the animals against the background of the bilateral occlusion of their common carotid arteries. This is probably due to the penetration intensification of the neuroprotector into the brain due to a decrease in the efflux activity of the transporter. Thus, the authors believe, they can speak about the expediency of reducing the functional activity of the ABCB1 protein in neurological diseases, the pathogenesis of which includes global cerebral ischemia.

CONCLUSION

The combination of the inhibitor and the ABCB1 protein substrate, omeprazole and nimodipine, respectively, does not increase the neuroprotective potential of the latter (the necrosis zone does not significantly decrease), and the ABCB1 protein inhibition cannot be considered as a promising aim to increase the effectiveness of pharmacotherapy in a focal cerebrovascular accident. This phenomenon is probably associated with a violation of the BBB integrity, in which the barrier functions of the transporter no longer play a significant role in the control of its substrates cerebral accumulation. However, such a tactic is justified in global cerebral ischemia, which is confirmed by a significant decrease in mortality and the level of neurological deficit in the animals with this pathology against the background of the combined use of these agents.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Ivan V. Chernykh – performing experiments on animals (modeling pathologies), article writing; Aleksey V. Shchulkin – statistical processing of the results obtained; Maria V. Gatsanoga – work with laboratory animals (drugs administration, brain sections staining, neurological deficit and mortality assessment); Elena N. Yakusheva – research design planning, article writing.

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