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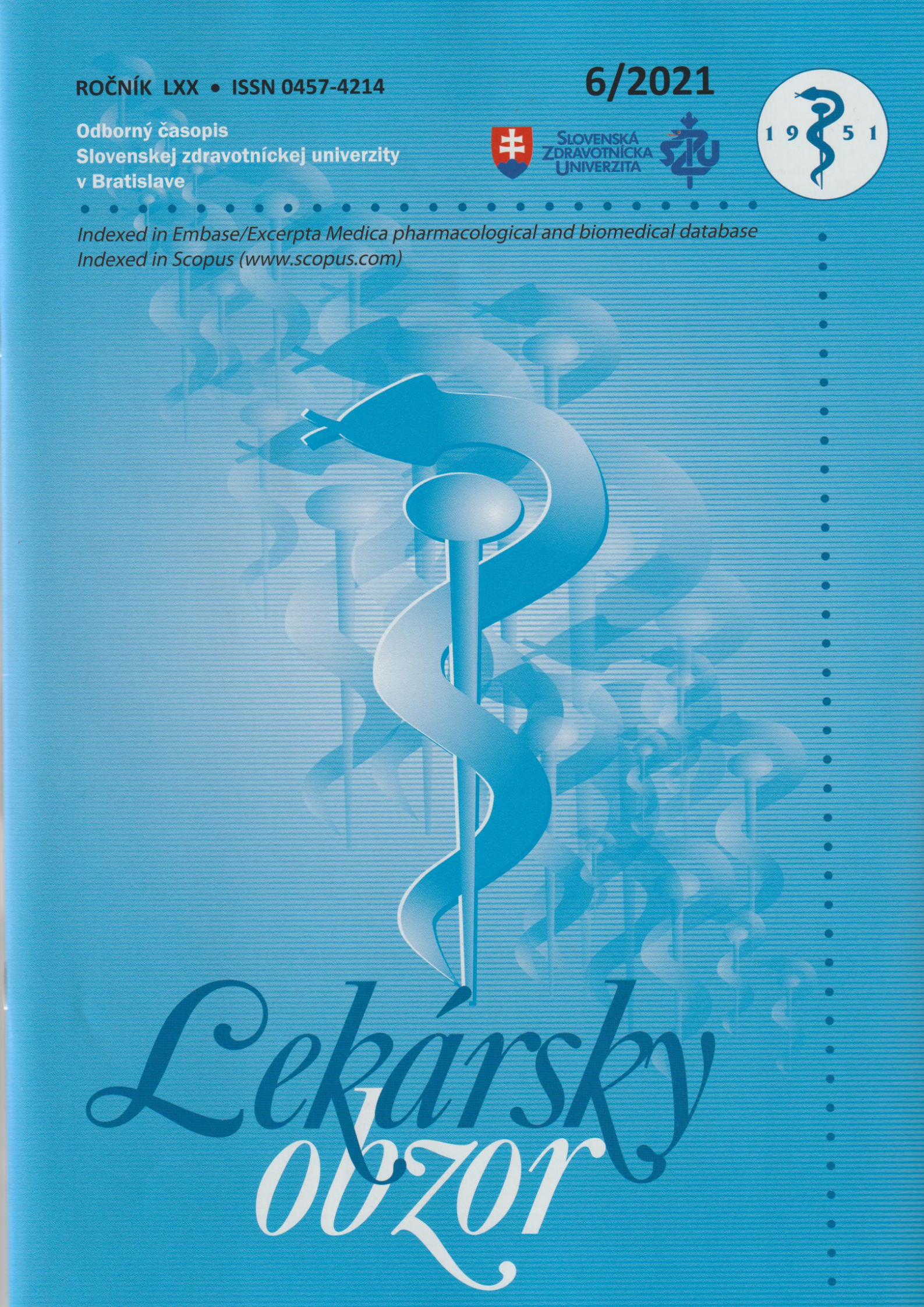

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THE INFLUENCE OF THE COURSE INFUSION OF ADEMOL ON THE POOL OF ADENYL NUCLEOTIDES AND INTERMEDIATES OF CARBOHYDRATE EXCHANGE IN THE BRAIN OF RATS WITH TRAUMATIC BRAIN INJURY

Vplyv infúznej kúry ademolu na pool adenylových nukleotidov a systém výmeny sacharidov v mozgu potkanov s traumatickým poškodením mozgu

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SUMMARY
The high expectations of modern medicine for neuroprotective therapy have encouraged scientists around the world to actively seek for new effective drugs. The effectiveness of neuroprotective agents is largely determined by their ability to normalize the metabolism of adenyl nucleotides and the associated biochemical processes in the brain. The aim of the study was to evaluate the magnitude of the cerebroprotective effect of ademol with the possibility of correcting the changes in the pool of adenyl nucleotides and carbohydrate metabolism intermediates in the brain of rats with experimental traumatic brain injury. The experimental model of severe trauma was caused by the action of a carbon dioxide flow under pressure, which was created with a gas-balloon air pistol. The therapeutic effect of ademol on model of traumatic brain injury was evaluated at a dose of 2 mg/kg i/v. The 8-days infusion of ademol solution in rats with traumatic brain injury at a conditionally effective cerebroprotective dose (2 mg/kg i/v) was more effective ($p<0.05$) than the use of amantadine sulfate (5 mg/kg i/v) and inhibited the hyperactivation of anaerobic glycolysis, stimulated tissue respiration, reduced the signs of lactic acidosis and the development of the secondary alteration of brain cells by deoxidized products.
Key words: ademol, adenyl nucleotides, carbohydrate exchange, rats, traumatic brain injury.
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Introduction

In connection with the war in the East of Ukraine, in recent years, there was a significant increase in both general injuries on the whole and brain injuries in particular (2). Today, traumatic brain injury (TBI) is the main and most common cause of disability and mortality of people aged 20 – 40 years, when the TBI mortality is 10 times higher than the one from cardiovascular diseases and 20 times higher than the one from malignant neoplasms (3, 6, 7, 9).

One of the triggers of brain damage is the violation of energy supply processes. The discrepancy of energy production in the system of mitochondrial oxidative phosphorylation to the energy needs of brain cells leads to the violation of numerous energy-dependent processes: membrane transport, synthesis of structural molecules, bioregulators and neurotransmitters, etc. (10).

The high hopes of modern medicine for neuroprotective therapy have stimulated the scientists around the world to actively search for new effective drugs which affect the pathophysiological cascades of neuronal damage (1, 3, 4). It is obvious that the effectiveness of neuroprotective agents is largely determined by their ability

to normalize the metabolism of adenyl nucleotides and associated biochemical processes in brain (5).

In view of this, there is considerable interest in investigating the effects of new cerebroprotectors on the state of energy metabolism at TBI.

The aim of the work is to evaluate the magnitude of the cerebroprotective action of ademol by the possibility of correcting changes in the pool of adenyl nucleotides and the intermediates of carbohydrate metabolism in the brain of rats with experimental TBI.

Materials and methods

Experiments were carried out on white male rats weighing 160 - 190 g, which were in the standard vivarium conditions, in compliance with the ethical standards of conducting experimental studies according to the General Principles of Work on Animals, approved by the I National Congress of Bioethics (Kyiv, Ukraine, 2001) and the Law of Ukraine „On Animal Protection from Cruel Treatment" of 26.02.2006. The experimental TBI model was caused by the action of carbon dioxide flow, which was created with a gas-balloon air pistol called „Baikal MP-654K" (RF, Izhevsk, certificate No. ROSS RU MG03.B02518) and the carbon dioxide balloons (liquefied CO_2 weight 12 g) under pressure (Crosman, USA, series No. 456739). The rats in condition of propofol anesthesia (60 mg/kg), after the catheterization of the femoral vein and making it possible to make an infusion through infusomate, performed right-sided osteoplastic trepanation of the skull of the middle cerebral artery projection, with a hole diameter of 5 mm^2. After fixing the rat in a position on the abdomen upside down, a shot was fired from a fixed distance (close-up shot), the bone fragment on the periosteum together with the aponeurosis was returned to the place and the wound was sutured in layers. Thus, severe TBI was simulated.

The therapeutic effect of Ademol (Ademol-Darnitsa, Darnitsa, Ukraine, 10 ampoules of 5 ml at a concentration of 1 mg/ml) on the model TBI was evaluated at a dose of 2 mg/kg intravenously. Treatment was happening via slow intravenous infusion using infusomate, which lasted 2 h with an interval of 2 t/d (every 12 h) for 8 days.

The treatment began 1 hour after modeling the pathological state. The pseudooperated animals were subjected to all interventions (anesthesia, skin incision, osteoplastic skull trepanation) with the exception of manipulations that could directly lead to traumatic brain damage, which offset the impact of the traumatic conditions of the experiment. They also had an equivalent amount of 0.9 % NaCl solution to the dose of Ademol injected. As drugs for the control group, we used a 0.9 % NaCl solution at a dose of 2 ml/kg i/v in the same mode, and for the comparison group we used amantadine sulfate („PC-Merz", Merz Pharmaceuticals, Switzerland, 200 mg/500 ml) on model TBI, which was evaluated at a dose of 5 mg/kg intravenously in the same mode.

For biochemical studies, after the euthanasia of animals, the rat brain was isolated, perfused with a cold 1.15 % potassium chloride solution and homogenized at 3000 rpm (Teflon glass) in the 1.15 % potassium chloride environment (1:3 ratio). The homogenates were centrifuged for 30 min at 600 g, the aliquots of the post-nuclear supernatant were taken into Erpendorf microtubes and stored at the temperature of -20 °C until the researches.

The content of adenyl nucleotides was determined in a protein-free trichloroacetic extract of brain tissues 1 : 10 (10 % solution of trichloroacetic acid) by the chromatographic method The content of pyruvate and lactate-calorimetric method of Asatiana V.S. (8). The energy charge was calculated by the formula:

$$\textbf{Energy charge} = (2ATP+ADP)/2(ATP+ADP+AMP)$$

The obtained results were processed by the method of variation statistics using the Student's t-test, changes in indicators were considered possible at $p \leq 0.05$.

Research results and their discussion

The results of the study showed that there are violations of energy metabolism in brain tissues and the development of hypoenergetic state in the animals of the control pathology group (Tab. 1).

Table 1. The effect of the course of 8-day infusion of ademol and amantadine sulfate on the content of adenyl nucleotide in the brain of rats with traumatic brain injury (M ± m, n = 7).

Groups of animals	Indicators			
	ATP, μmol/g of dry tissue	ADP, μmol/g of dry tissue	AMP, μmol/g of dry tissue	Energy charge
Pseudoperated animals + 0.9% NaCl solution	3.13 ±0.49	0.957 ±0.018	0.667 ±0.022	0.767 ±0.006
TBI + 0.9 % NaCl solution (control pathology)	1.66 ± 0.07°	2.20 ±0.07°	1.06 ±0.03°	0.560 ±0.006°
TBI + ademol, 2 mg/kg i/v	3.10 ±0.12*ǂ	0.943 ±0.012 *ǂ	0.639 ±0.032 *ǂ	0.763 ±0.004*ǂ
TBI +amantadine sulfate, 5 mg/kg i/v	2.52 ±0.11ş*	1.40 ±0.04ş*	0.737 ±0.014ş*	0.691 ±0.006ş*

TBI - traumatic brain injury; ^ - $p < 0.05$ regarding pseudo-operated animals; * - $p < 0.05$ regarding the control pathology group; ǂ - $p < 0.05$ regarding amantadine sulfate (5 mg/kg i/v). Source: Authors´ own processing.

In pseudo-operated rats the ATP level in brain tissues was in the range of 1.50 - 4.73 μmol/g of dry tissue, the ADP content - 0.904 - 1.02 μmol/g of dry tissue, the AMP concentration - 0.608 - 0.743 μmol/g of dry tissue and the energy charge (P_5-P_{95}). At the same time, the untreated animals with TBI had less ATP in brain by 47.1 % ($p < 0.05$), compared to pseudo-operated animals, and ranged from 1.44 to 1.88 μmol/g of dry tissue (P_5-P_{95}). Under these conditions, the content of ADP and AMP exceeded the pseudo-operated animals' indicators by 130 and 58.9 % ($p < 0.05$), and varied accord-

ingly between 1.94 - 2.43 μmol/g of dry tissue and 0.95 - 1.14 μmol/g of dry tissue (P_5-P_{95}). In addition, after TBI the ratio between adenyl nucleotides changed, as evidenced by a probable drop in energy charge by 73.0 % ($p < 0.05$). So, in pseudo-operated animals, the energy charge changed within 0.753 - 0.786, and in animals of the control pathology group were in the range 0.547 - 0.579 (P_5-P_{95}).

The obtained results confirm the formation of a hypoenergetic state in the brain cells of rats on the background of TBI, which is probably the consequence of the inhibition of oxidative phosphorylation and its conjugation with tissue respiration.

The use of the studied drugs inhibited the formation of energy-deficient state in the brain in experimental conditions, improved the process of oxidative phosphorylation and its conjugation with tissue respiration, but the expression of their effects differed significantly. It was found that the course infusion of ademol prevented perturbations in the metabolism of adenyl nucleotides to the greatest extent. Under these conditions, the ATP level in the brain and the energy charge were 85.3 and 36.3 % ($p < 0.05$) higher compared to the pseudooperated animals, and ranged from 2.77 to 3.58 μmol/g of dry tissue (P_5-P_{95}) and 0.751-0.775 respectively.

The levels of ADP and AMP in the brain were lower by 57.1 and 39.7 % ($p < 0.05$), compared to the pseudooperated animals, and varied between 0.911 - 0.988 and 0.522 - 0.745 μmol/g of dry tissue (P_5-P_{95}), respectively. By the efficiency of adjusting the energy metabolism in the brain of rats, the reference drug amantadine sulfate was significantly inferior to ademol. In the group of animals treated with amantadine, the ATP level and energy charge were 52.4 and 23.4 % ($p < 0.05$) higher compared to the pseudooperated animals, and varied in the diapason of 2.13 - 2.87 μmol/g of dry tissue (P_5-P_{95}) and 0.674 - 0.709, respectively. Under these conditions, the concentrations of ADP and AMP in the brain were lower by 36,6 and 30.5 % ($p < 0.05$), compared to the pseudooperated animals, and varied between 1.28 - 1.51 and 0.686 - 0.7772 μmol/g of dry tissue (P_5-P_{95}), respectively.

The results of our studies confirm the fact that TBI is accompanied by the activation of anaerobic glycolysis and the inhibition of the aerobic oxidation of glucose, as evidenced by the imbalance between the level of intermediates lactate and pyruvate (Tab. 2). In pseudooperated animals, the level of lactate varies between 1.68 - 1.98 μmol/g of dry tissue (P_5-P_{95}), pyruvate - in the range of 0.255 - 0.417 μmol/g of dry tissue (P_5-P_{95}), and the ratio of lactate/pyruvate is in the range of 3.94 - 6.96. Instead, at TBI there is a statistically probable increase in lactate levels by 3.58 times (in the range of 6.55 - 7.47 μmol/g of dry tissue), a decrease in pyruvate content by 56.2 % (ranging from 0.122 to 0.178 μmol/g of dry tissue) and an increase in the ratio of lactate/pyruvate by 8.64 times (ranging from 36.1 to 52.9), compared to the pseudooperated animals. Numerous experimental studies have shown that the formation of ATP deficiency and excess di- and triphosphate nucleosides in brain structures at TBI causes the activation of the regulatory enzymes of anaerobic glycolysis by an allosteric mechanism. The hyperactivation of glycolysis is accompanied by the increased production of lactic acid, the development of metabolic acidosis, which deepens the alteration of brain cells at TBI.

The results of our studies confirm the fact that TBI is accompanied by the activation of anaerobic glycolysis and the inhibition of the aerobic oxidation of glucose, as evidenced by the imbalance between the level of intermediates lactate and pyruvate (Table 2). In pseudooperated animals, the level of lactate varies between 1.68 - 1.98 μmol/g of dry tissue (P_5-P_{95}), pyruvate - in the range of 0.255 - 0.417 μmol/g of dry tissue (P_5-P_{95}), and the ratio of lactate/pyruvate is in the range of 3.94 - 6.96. Instead, at TBI there is a statistically probable increase in lactate levels by 3.58 times (in the range of 6.55 - 7.47 μmol/g of dry tissue), a decrease in pyruvate content by 56.2 % (ranging from 0.122 - 0.178 μmol/g of dry tissue) and an increase in the ratio of lactate/pyruvate by 8.64 times (ranging from 36.1 to 52.9), compared to the pseudooperated animals.

The use of the studied cerebroprotectors, especially ademol, reduced the activity of anaerobic glycolysis, promoted the activation of aerobic glucose oxidation and reduced the signs of lactic acidosis in brain cells at TBI (Tab. 2).

Tab. 2. The effect of the course 8-day infusion of ademol and amantadine sulfate on the content of glucose metabolites in the brain of rats with traumatic brain injury (M±m, n=7).

Groups of animals	Indicators		
	Lactate, μmol/g of dry tissue	Pyruvate, μmol/g of dry tissue	Lactate/ Pyruvate
Pseudoperated animals + 0.9% NaCl solution	1.82±0.05	0.347±0.025	5.45±0.45
TBI + 0.9% NaCl solution (control pathology)	7.02±0.14°	0.152±0.008°	47.1±2.94°
TBI + ademol, 2 mg/kg i/v	2.60±0.08°*ǂ	0.321±0.009*	8.13±0.24°*ǂ
TBI + amantadine sulfate, 5 mg/kg i/v	3.42±0.24°*	0.317±0.012°*	10.9±0.84°*

TBI - traumatic brain injury; ş - $p < 0.05$ regarding pseudooperated animals; * - $p < 0.05$ regarding the group of control pathology; ǂ - $p < 0.05$ regarding amantadine sulfate (5 mg/kg i/v). Source: Authors´ own processing.

It is shown that the use of ademol inhibited the disorders of the aerobic oxidation of glucose on the background of TBI quite effectively. Under these conditions, the level of lactate was 2.7 times lower (ranged from 2.31 to 2.88 μmol/g of dry tissue), the concentration of pyruvate was higher by 111 % (varied in the range of

0.297 - 0.350 μmol/g of dry tissue), and the ratio of lactate/pyruvate was lower by 5.8 times (in the range of 7.34 - 8.96), compared with the corresponding indicators in the group of untreated animals with TBI. The use of amantadine sulfate in a less effective way than ademol, affected the processes of aerobic carbohydrate metabolism. In the group of animals treated with an 8-day infusion of amantadine, there was a probable decrease in lactic acid content by 2.1 times (its level ranged from 2.57 to 4.22 μmol/g of dry tissue), an increase in pyruvate by 109 % (it was in the range of 0.274 - 0.357 μmol/g of dry tissue) and a decrease in the ratio of lactate/pyruvate by 4.3 times (ranged from 7.86 to 13.7), compared to the animals in the control pathology group.

So, among the molecular mechanisms of the cerebroprotective activity of ademol and amantadine sulfate, we should note their ability to reduce energy deficiency and improve the aerobic metabolism of glucose in rat brain cells on the background of traumatic brain injury.

Conclusions and prospects for further development

1. The use of amantadine sulfate in rats with TBI significantly better corrects carbohydrate metabolism disorders than the 0.9 % NaCl solution and helps to reduce the metabolic acidosis manifestations in the damaged brain ($p < 0.05$).
2. Eight-day infusion in rats with traumatic brain injury of ademol solution in a conditionally effective cerebroprotective dose (2 mg/kg i/v) is more effective than the use of amantadine sulfate (5 mg/kg i/v) and inhibits the hyperactivation of anaerobic glycolysis, stimulates tissue respiration processes, reduces the signs of lactate acidosis and the development of the secondary alteration of brain cells by deoxidized products.

The therapeutic effect that was obtained during the experiment from the use of ademol is the basis for studying the protective effect of other cerebroprotectors at TBI.*

*__Conflict of interest:__ Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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