# EFFECTIVENESS OF CORRECTION OF DISCUSSION OF CARBOHYDRATE METABOLISM IN RATS WITH CRANIOCEREBRAL INJURY

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**Abstract:** The development and introduction into practice of emergency neurology of new drugs capable of influencing secondary neuronal damage in patients with traumatic brain injury (TBI) has made a significant impact on the recovery of such patients.

**Aim of the work** was to evaluate the magnitude of cerebroprotective action of amantadine sulfate in terms of impaired carbohydrate metabolism of the brain against the background of experimental TBI.

**Materials and methods**. The experimental model of severe TBI was caused by the action of a carbon dioxide flow under pressure, which was created using a gas balloon of pneumatic gun. The therapeutic effect of amantadine sulfate on TBI model was evaluated with a 5 mg/kg dose. The pseudo-operated animals and control group received 0.9 % NaCl solution at a dose of 2 ml/kg with IV administration.

**Results**. The obtained results confirm the formation of a hypo-energetic state in the brain cells of rats on the background of TBI, which is probably a consequence of the suppression of the processes of oxidative phosphorylation and its conjugation with tissue respiration. TBI is accompanied by activation of anaerobic glycolysis and inhibition of aerobic glucose oxidation, proving that there is an imbalance between the levels of lactate and pyruvate intermediates. 8-day infusion of rats with traumatic brain injury of amantadine sulfate, inhibited anaerobic glycolysis hyperactivation, stimulated tissue respiration processes, reduced signs of lactic acidosis and development of secondary alteration of brain cells by non-oxidized products.

**Conclusions**. In the course of the experiment, it was found that the treatment of rats, with TBI model, with 0.9 % NaCl solution did not significantly contribute to the restoration of impaired energy metabolism ($p<0.05$) and did not reduce the manifestation of metabolic acidosis in the damaged brain ($p<0.05$). The use of amantadine sulphate in TBI rats significantly corrects carbohydrate metabolism disorders than 0.9 % NaCl solution and contributes to the reduction of metabolic acidosis in the damaged brain ($p<0.05$).

**Keywords:** traumatic brain injury, amantadine sulfate, rats.

## 1. Introduction

Development and implementation of emergency neurology and neurosurgical practice of new drugs capable of influencing secondary damage of neurons in patients with traumatic brain injury (TBI), allowed to significantly influence the recovery of such patients, reduce the duration of stay in therapy department; improve cognitive function rehabilitation and recovery. The high expectations of modern medicine for neuroprotective therapy have stimulated scientists all over the world to actively search for new effective drugs of influencing the pathophysiological cascades of neuronal damage development [1, 2].

One of the trigger mechanisms for brain damage is the disruption of energy supply. The mismatch of energy production in the system of mitochondrial oxidative phosphorylation of the energy consumption of brain cells leads to disruption of numerous energy dependent processes: membrane transport, synthesis of structural molecules, bioregulators and neurotransmitters, etc. [3]. It is quite clear that the effectiveness of neuroprotective agents is largely determined by their ability to normalize the metabolism of adenylated nucleotides and the associated biochemical processes in the brain [4].

Today, the main component of intensive care for TBI is therapy, which includes a number of interventions aimed at preventing the development and correcting the manifestations of homeostasis disorders that go along with traumatic brain damage. According to modern ideas, cerebroprotectors can perform adequate protection of the brain against the background of traumatic damage [5, 6]. One of the complications accompanying TBI is acute cerebral ischemia [6]. The choice of medicines (drugs) for brain injuries is one of the most complex problems in the treatment of such patients [7].

Considering this, it is of great interest to study the effect of various cerebroprotectors on the state of energy metabolism in TBI.

The aim of the study was to evaluate the magnitude of the cerebroprotective effect of amantadine sulfate on the possibility of pharmacological correction of carbohydrate metabolism disorders in the rat brain against experimental TBI.

## 2. Materials and methods

The studies were conducted at the Vinnytsia National Medical University for the period 2016–2019.

The experiments were performed on white male rats weighing 160–190 g, who were in the vivarium of the VNMU named after M.I. Pirogov on a standard water and food ration (chilled boiled water, fodder grain, bread, granulated feed, temperature 20–24 °C, humidity 50–55 %) in natural light 12 hours a day and free access to water and feed, in compliance with the ethical standards of experimental research in accordance with the "General principles of work on animals", approved by the First National Congress on Bioethics (Kyiv, Ukraine, 2001) and the Law of Ukraine "On the Protection of Animals against Cruelty" of 26.02.2006. TBI experimental model was caused by action of flow of carbon dioxide under pressure created with the use of a gas cylinder of pneumatic gun brand "Baikal MR-654K" (RF, Izhevsk, No. ROSS certificate RU MZH03.V02518) and carbon dioxide cylinders (mass of liquefied $CO_2$ (12 gm), under pressure of $CO_2$ – 12 g) USA, lot number 456739). Rats under conditions of propofol anesthesia (60 mg/kg), after catheterization of the femoral vein and the possibility of infusion through the pump system, performed right-sided bone-plastic trepanation of the skull of the projection of the middle cerebral artery, with a hole diameter of 5 $mm^2$. After fixation of the rat in a position on the abdomen upside down, a shot was taken from a fixed distance (close-up shot), the bone fragment on the periosteum together with aponeurosis was returned to the site and the wound was sutured in layers. Thus, the TBI was heavily modelled.

The therapeutic effect of amantadine sulfate (PC-Merz, Merz Pharmaceuticals, Switzerland), 1 vial of 500 ml at a concentration of 200 mg/500 ml on model TBI was evaluated by the administration of a dose of 5 mg/kg intravenously. The treatment was performed by slow intravenous (I/V) infusion with infusion pump at intervals of 2 g/d (every 12 h) for

8 days. Treatment was started 1 hour after the simulation of the pathological condition. The pseudo-operated animals were subjected to all interventions (anaesthesia, skin incision, bone-plastic skull trepanation) with the exception of manipulations that could directly lead to traumatic brain injury, which nullified the effects of trauma. They were also administered an equivalent amount of 0.9 % NaCl solution up to a dose of amantadine sulfate. As a drug for the control group used 0.9 % solution of HCl at a dose of 2 ml/kg in/in the same mode.

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

The content of adenyl nucleotides was determined in protein-free trichloroacetic extract of brain tissues 1:10 (10 % trichloroacetic acid solution) by chromatographic method. Content of pyruvate and lactate-calorimetric method [9]. The energy charge was calculated by the formula:

$$Energy\ charge = \frac{2ATP + ADP}{2(ATP + ADP + AMP)}.$$

The obtained results were processed by the method of variational statistics using Student's t-test, changes of indicators were considered reliable at p≤0.05.

It has been established that the course administration of amantadine sulfate somewhat prevented perturbations in the metabolism of adenyl nucleotides. In the group of animals treated with amantadine, the ATP content and energy charge were 52.4 and 23.4 % (p<0.05) higher, compared to the pseudoperated animals, and varied in the range 2.13–2.87 μmol/g dry cloth (P5-P95) 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 with pseudoperated animals, and varied between 1.28–1.51 and 0.686–0.7772 μmol/g of dry tissue (P5-P95), respectively.

**Table 1**

Effect of a 8-day course infusion of amantadine sulfate on the content of adenyl nucleotides in the brain of rats with traumatic brain injury (M±m, n=7)

| Group of animals | Indicators | | | |
|---|---|---|---|---|
| | ATP, μmol/g of dry tissue | ADP, μmol/g of dry tissue | AMP, μmol/g of dry tissue | Energy charge |
| Pseudo-operated 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± 0.07º | 2.20±0.07º | 1.06± 0.03º | 0.560±0.006º |
| TBI + amantadine sulfate, 5 mg / kg | 2.52±0.11º* | 1.40±0.04º* | 0.737±0.014º* | 0.691±0.006º* |

*Note: TBI – traumatic brain injury; º – p<0.05 relative to pseudo-operated animals; * – p<0.05 relative to the control pathology group*

## 3. Results

Disruption of energy metabolism in traumatically damaged neurons is one of the main pathogenetic mechanisms for the development of neurodegradative changes in TBI. Therefore, it was advisable to evaluate the state of energy metabolism in the tissues of the brain of rats by TBI and based on 8-day therapy course with amantadine sulfate.

The results of the study showed that the animals of the control pathology group observed disorders of energy metabolism in brain tissues and develops a hypo-energetic state (**Table 1**). In pseudo-operated rats in brain tissues, the ATP level was in the range of 1.50–4.73 μmol/g of dry tissue, the ADP content was 0.904–1.02 μmol/g of dry tissue, the concentration of AMP was 0.608–0.743 μmol/g of dry tissue, and the energy charge (P5-P95). At the same time, in untreated TBI animals, the ATP level in the brain was lower by 47.1 % (p<0.05), compared to the pseudo-operated animals, and ranged from 1.44 to 1.88 μmol/g of dry tissue (P5-P95). Under these conditions, the content of ADP and AMP exceeded the values of pseudo-operated animals by 130 and 58.9 % (p<0.05), and varied respectively within 1.94–2.43 μmol/g of dry tissue and 0.95–1.14 μmol/g of dry tissue (P5-P95). In addition, the CCI changed the ratio between adenyl nucleotides, as evidenced by a likely decrease in energy charge of 73.0 % (p<0.05). Thus, in pseudo-operated animals the energy charge varied within 0.753–0.786, and in animals of the control pathology group was in the range 0.547–0.579 (P5-P95).

The results confirm the formation of a hypo-energetic state in the brain cells of rats on the background of TBI, which is probably a consequence of the suppression of the processes of oxidative phosphorylation and its conjugation with tissue respiration.

Numerous experimental studies have shown that the formation of ATP deficiency and excess of di- and three phosphate nucleosides in brain structures by TBI causes activation of the anaerobic glycolysis regulatory enzymes by the allosteric mechanism. Hyperactivation of glycolysis was accompanied by increased production of lactic acid, the development of metabolic acidosis, which deepens the alteration of brain cells by TBI.

The results of our studies confirm the fact that TBI was accompanied by activation of anaerobic glycolysis and inhibition of aerobic glucose oxidation, which is evidence of an imbalance between the levels of lactate and pyruvate intermediates. In pseudo-operated animals, the level of lactate varies within 1.68–1.98 μmol/g of dry tissue (P5-P95), pyruvate – in the range of 0.255–0.417 μmol/g of dry tissue (P5-P95), and the ratio of lactate/pyruvate was in the range of 3.94–6.96. Instead, a statistically significant increase in lactate levels of 3.58-fold (in the range of 6.55–7.47 μmol/g of dry tissue) was observed with TBI, a decrease in pyruvate content of 56.2 % (ranges from 0.122–0.178 μmol/g of dry tissue) and an increase of lactate/pyruvate ratio of 8.64 times compared to pseudo-operated animals.

The use of amantadine sulfate decreased the activity of anaerobic glycolysis, promoted the activation of aerobic glucose oxidation and reduced the signs of lactic acidosis in brain cells by TBI (**Table 2**). The use of amantadine sulfate effectively influenced the processes of aerobic metabolism of carbohydrates. In the group of animals administered 8-day infusion of amantadine, a significant decrease in lactic acid content by 2.1 times (its level ranged from 2.57–4.22 μmol/g of dry tissue), a pyruvate increase of 109 % (was in the range of 0.274–0.357 μmol/g dry tissue) and a decrease in the ratio of lactate / pyruvate 4.3 times, compared with the animals in the control group of pathology.

**Table 2**

Effect of 8-day infusion course of amantadine sulfate on the content of glucose metabolites in the brain of rats with traumatic brain injury (M±m, n=7)

| Group of animals | Indicators | | |
|---|---|---|---|
| | Lactate, μmol/g of dry tissue | Pyruvate, μmol/g of dry tissue | Lactate / Pyruvate |
| Pseudo-operated 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$^{o}$ | 0.152±0.008$^{o}$ | 47.1±2.94$^{o}$ |
| TBI + amantadine sulfate, 5 mg / kg | 3.42±0.24$^{o*}$ | 0.317±0.012$^{o*}$ | 10.9±0.84$^{o*}$ |

*Note: TBI – traumatic brain injury; $^{o}$ – p<0.05 relative to pseudo-operated animals; * – p<0.05 relative to the control pathology group*

## 4. Discussion and conclusions

In 2014, B. Elkon, J. R. Cambrin, E. Hirshberg, S. L. Bratton published the results of his own study examining the effect of blood glucose levels on the effects of TBI in children [10]. High blood glucose was associated with a 3.5-fold increase in poor prognosis compared to normal glycemic levels, adjusted for injury severity and cardiovascular instability [10].

The data obtained in our study indicate that 8-day infusion of rats with brain injury of amantadine sulfate, inhibited anaerobic glycolysis hyperactivation, stimulated tissue respiration processes, and reduced signs of lactic acidosis and development of secondary motility. Therefore, among the molecular mechanisms of cerebroprotective activity of amantadine sulfate should be highlighted its ability to reduce energy deficiency and improve aerobic glucose metabolism in rat brain cells against a background of traumatic brain injury.

Of considerable interest to the scientific community as well as to practical medicine are the results of the analysis of 104 studies on the effects of hyperglycemia on the prognosis of adult patients with TBI, conducted by J. Peffer, C. McLaughlin [11]. Among the publications included in this study, the overwhelming majority determined the negative impact of elevated glucose levels on the prognosis of patients with brain injury.

Compared with pseudo-operated animals, treatment of rats with a model TBI of 0.9 % NaCl solution did not significantly restore impaired energy metabolism ($p<0.05$) and did not reduce the manifestation of metabolic acidosis in the injured brain ($p<0.05$).

The use of amantadine sulphate in TBI rats significantly corrects carbohydrate metabolism disorders than 0.9 % NaCl solution and contributes to the reduction of metabolic acidosis in the damaged brain ($p<0.05$).

The therapeutic effect, which was obtained in the experiment from therapy with amantadine sulfate, is the basis for studying the protective effect of other cerebroprotectors in treatment of TBI.

## Conflict of interests

No conflict of interest.

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