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Semenenko S.I.
National Pirogov Memorial Medical University, Vinnytsia, Ukraine
Khrebtii H.I.
Higher Education Institution in Ukraine "Bukovinian State Medical University", Chernivtsi
Semenenko A.I.
National Pirogov Memorial Medical University, Vinnytsia, Ukraine

INVESTIGATION OF THE IMPACT OF AMANTADINE SULPHATE ON CERTAIN PATHOGENETIC MECHANISMS OF TRAUMATIC BRAIN INJURY

Abstract. *The goal of the work to characterize the effect of amantadine sulfate compared with 0.9% NaCl solution on the course of oxidative stress in the brain of rats with traumatic brain injury. The experiments were performed on white male rats weighing 160-190 g. Treatment of rats with TBI with amantadine sulfate leads to a decrease in the activity of lipid peroxidation processes and oxidative destruction of proteins ($p<0,05$) and helps to normalize the activity of antioxidant enzymes in cells of traumatically damaged brain ($p<0,05$). The use of amantadine sulfate compared with 0,9% NaCl solution was accompanied by a more significant decrease in the activity of lipid peroxidation processes and oxidative destruction of proteins and an improvement in the level of antioxidant enzymes in damaged brain of animals with TBI ($p<0,05$).*

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

Introduction. The analysis of the literature indicates that, in the case of brain damage, hyperproduction of reactive oxygen species by brain tissues is registered, which activates the development of oxidative and nitrosative stress of proteins, lipids and nucleic acids of neurons in its structures, violates the affinity and specificity of receptors, generation of action potentials and conduction of nerve impulse [1, 2]. Therefore, the use of pharmacotherapeutic agents with antioxidant properties is an important measure of intensive care in patients with traumatic brain injury (TBI) [3]. Many studies on the treatment of nosology accompanied by both traumatic and ischemic brain damage have demonstrated the efficacy of glutamate NMDA receptor antagonists. Blockade of NMDA receptors is considered to be one of the main links in neuroprotection [4, 5, 6]. That is why it was expedient to investigate the effect of amantadine sulfate in a difficult TBI experimental model on the course of TBI oxidative stress as a drug capable of blocking NMDA receptors.

Materials and methods. The experiments were performed on white male rats weighing 160-190 g, which were in standard vivarium conditions, in accordance with the ethical standards of experimental studies. Rats under conditions of propofol anesthesia were performed right-sided bone-plastic trepanation of the skull in the projection of the middle cerebral artery and simulated severe TBI. The therapeutic effect of amantadine sulfate ("PC-Mertz", Merz Pharmaceuticals, Switzerland) at a concentration of 200 mg / 500 ml on model TBI was evaluated by the administration of a dose of 5 mg / kg intravenously. Treatment was performed by slow intravenous (i/v) infusion with infusomate every 12 h for 8 days. Treatment was started 1 h after simulation of the pathological condition. Pseudoperated animals were subjected to all interventions (anesthesia, skin incision, skeletal bone-plastic trepanation) with the exception of manipulations that could directly lead to traumatic brain injury, which eliminated the effects of traumatic conditions of the experiment. 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 we used 0.9% NaCl solution at a dose of 2

ml / kg i/v the same mode.

Biochemical processes in traumatically damaged brain were investigated on the 8th day of TBI. The content of malondialdehyde (MDA) - by reaction with thiobarbituric acid, carbonyl groups of proteins (CGP) - by reaction with 2,4-dinitrophenylhydrazine. The activity of superoxide dismutase (SOD) was evaluated by the percentage of inhibition of quercetin oxidation, and catalase by the rate of degradation of hydrogen peroxide. The activity of glutathione peroxidase (GPO) was determined by spectrophotometric method with the accumulation of oxidized glutathione [7]. Quantitative data were processed using the StatPlus 2009 statistical processing program.

Results and Discussion. Nowadays, many current experimental and clinical studies are aimed at finding effective molecules that can prevent secondary neuronal damage caused by the multifaceted pathogenetic mechanism of brain damage in TBI. The action of NMDA receptor blockers is aimed at inhibiting the rapid responses of the glutamate calcium cascade, which can reduce secondary damage to neurons against TBI [5, 6]. Under the conditions of experimental TBI, it was found that hyperactivation of free radical oxidation of biomembrane lipids is registered in the structures of the brain of rats (fig. 1). In the group of pseudoperated animals, the median content of the secondary metabolite of malone dialdehyde (MDA) in the brain is 13,2 (95% CI 12,8-14,2) µmol / g of dry tissue, and the percent interval P_{25}-P_{75} - 13,1-14,0 µmol / g of dry tissue. At the same time, in animals of the control pathology group, this indicator is 2,28 times ($p<0,05$) higher than in pseudoperated animals, its median is equal to 30,8 (95% CI 28,6-33,3) µmol / g of dry tissue, and the percentile interval P_{25}-P_{75} is in the range of 29,4-31,8 µmol / g of dry tissue. In the ability to inhibit lipid peroxidation, amantadine sulfate was superior to the TBI + NaCl group. In animals treated with amantadine sulfate, the MDA content in the brain was lower by 48,4% ($p<0,05$) than in the animals of the control pathology group, the median was 16,1 (95% CI 14,9-16,7) µmol / g of dry tissue, and the percentile interval P_{25}-P_{75} of 15,5-16,3 µmol / g of dry tissue.

The development of TBI is associated with the activation of oxidative modification of proteins (fig. 2). In pseudoperated animals, the median content of carbonyl groups of proteins (CGP) in the brain is 4,73 (95% CI 4,29-5,01) µmol / g of dry tissue, and the percentile interval P_{25}-P_{75} is 4,57-4,94 µmol / g of dry tissue. On the other hand, at TBI the level of carbonylated proteins was 1,77 times ($p<0,05$) higher than in pseudoperated animals, its median was 8,66 (95% CI 7,89-9,67) µmol / g of dry tissue, and the percentile interval P_{25}-P_{75} was in the range of 8.1,7-9,28 µmol / g of dry tissue. The use of amantadine sulfate inhibits

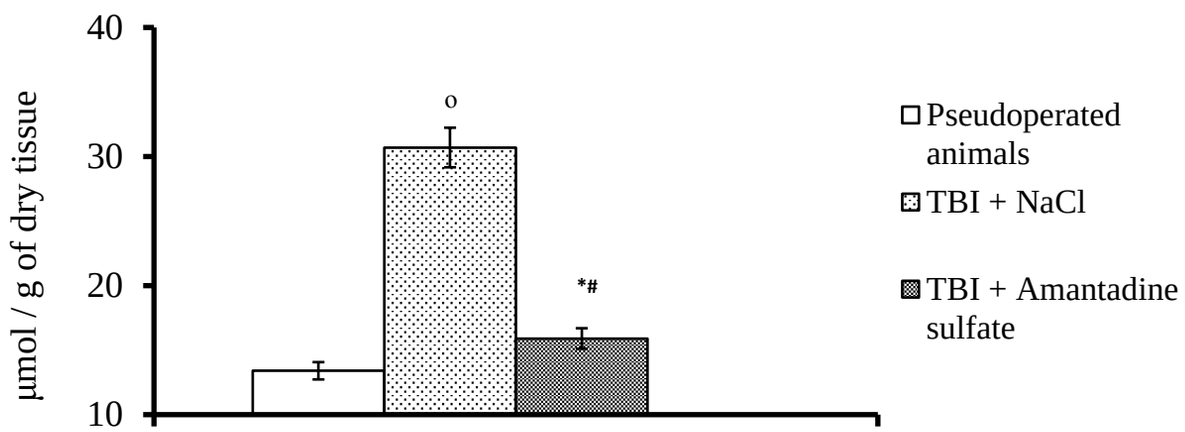

Fig. 1. Effect of a course 8-day infusion of amantadine sulfate on the content of lipid peroxidation products in the brain of rats with TBI (M±m, n=7).

Notes:
TBI - traumatic brain injury;
º – p<0,05 relative to pseudoperated animals;
** - p<0,05 relative to the control pathology group*

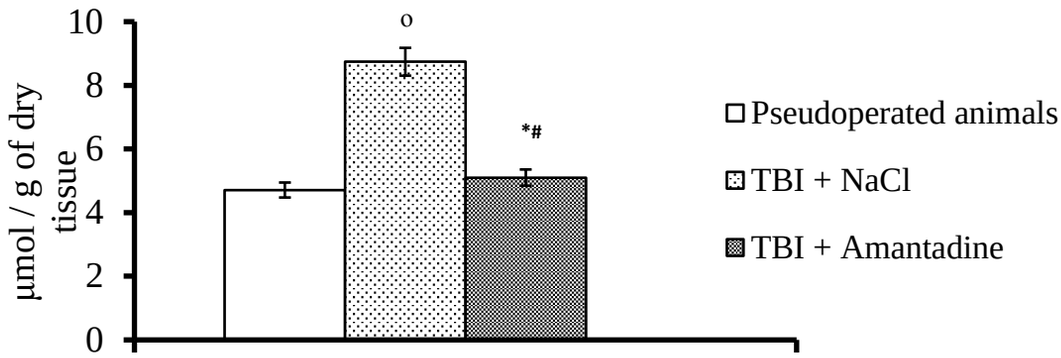

Fig. 2. Effect of a course 8-day infusion of amantadine sulfate on the content of protein peroxidation products in the brain of rats with TBI (M±m, n=7).

Notes:
TBI - traumatic brain injury;
º – p<0,05 relative to pseudoperated animals;
** - p<0,05 relative to the control pathology group.*

the activation of the processes of oxidative degradation of proteins in brain tissues. Thus, in animals treated with amantadine sulfate, the CGP content in the brain was 39,1% lower (p<0,05) than in the animals of the control pathology group, the median was 4,99 (95% CI 4,65-5,59) µmol / g of dry tissue, and the percentile interval P_{25}-P_{75} - 4,83-5,39 µmol / g of dry tissue.

Thus, the use of amantadine sulfate at TBI prevents the hyperactivation of the processes of free radical oxidation of lipids and proteins of brain cell biomembranes.

Hyperactivation of peroxidation of lipids and proteins at TBI occurs against the background of a decrease in the rate of inactivation of the superoxide anion radical. It is established that the median activity of the superoxide dismutation reaction involving SOD in the brain of pseudoperated animals is 2,68 (95% CI 2,23-3,05) c.u / mg of protein, and P_{25}-P_{75} – 2,50-2,87 c.u / mg of protein. At the same time, at TBI a probable decrease of SOD activity in the brain by 51,7% (p<0,05) is registered, the median activity of this enzyme is 1,31 (95% CI 0,97-1,57) c.u / mg of protein, and P_{25}-P_{75} – 1,22-1,40 c.u / mg of protein.

Applied pharmacotherapy prevented a decrease in the rate of superoxide anion dismutation reaction in the brain of animals with TBI. In animals treated with amantadine sulfate, SOD activity in the brain was 101% less, its median activity was 2,53 (95% CI 2,09-3,11) c.u / mg of protein, and P_{25}-P_{75} - 2 ,28-2,92 c.u / mg of protein.

TBI is accompanied not only by disruption of superoxide anion dismutation in brain cells, but also by inhibition of hydrogen peroxide inactivation with the participation of GPO and catalase enzymes (table 4). In the group of pseudoperated animals, the activity of the GPO varies within 69,3-77,9 µmol / min per 1 mg of protein, and the activity of catalase in the range of 5,94-7,59 µkat / mg of protein. However, the development of TBI causes a probable decrease of GPO activity in brain by 70,9% (its activity varies within 32,8-47,2 µmol / min per 1 mg of protein) and catalase by 46,9% (its activity varies within 3,01-4,16 µkat / mg of protein).

The pharmacotherapy with the study drug restrained the fall in the activity of the antioxidant enzymes GPO and catalase in brain structures (table). In animals treated with amantadine sulfate, the activity of GPO in brain tissues was higher by 44,5% (ranged from 55,5 to 61,2 µmol / min per 1 mg of protein) and catalase activity by 79,0% (varied from 6,21 to 6,75 µkat / mg of protein) than the corresponding indicators in the control pathology group.

According to the results of our studies, we have shown that the use of amantadine sulfate on the background of TBI restrains the fall of the activity of the enzymatic antioxidant link, prevents the accumulation of active oxygen intermediates and slows down the course of reactions of free radical oxidation of lipids and proteins in structures of rats' brain.

Table

The effect of a course of 8-day infusion of amantadine sulfate on the activity of antioxidant enzymes in the brain of rats with traumatic brain injury (M±m, n=7)

Groups of animals	Enzyme activity	
	GPO, µmol / min per 1 mg of protein	Catalase, µkat / mg of protein
Pseudoperated animals + 0.9% NaCl solution	72,7±1,37	6,82±0,26
TBI + 0.9% NaCl solution (control pathology)	40,2±2,30º	3,62±0,17º
TBI + amantadine sulfate, 5 mg / kg i/v	58,1±0,84º*	6,48±0,08º*

Notes:

1. *TBI - traumatic brain injury;*
2. *º - p<0.05 relative to pseudoporous animals;*
3. ** - p<0.05 relative to the control pathology group.*

Conclusions:

1. Treatment of rats with TBI with amantadine sulfate leads to a decrease in the activity of lipid peroxidation processes and oxidative destruction of proteins (p<0,05) and helps to normalize the activity of antioxidant enzymes in cells of traumatically damaged brain (p<0,05).

2. The use of amantadine sulfate compared with 0,9% NaCl solution was accompanied by a more significant decrease in the activity of lipid peroxidation processes and oxidative destruction of proteins and an improvement in the level of antioxidant enzymes in damaged brain of animals with TBI (p<0,05).

The prospect of further researches. The therapeutic effect obtained in the experiment from the therapy of amantadine sulfate is the basis for a more in-depth study of the protective effect on the brain at TBI of other NMDA receptor blockers..

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