Short Research Article

STUDY OF THE EFFECT OF ADEMOL ON THE DYNAMICS OF S 100 PROTEIN IN RATS WITH TRAUMATIC BRAIN INJURY

Abstract

Currently, the problem of choosing an effective drug approach for cerebroprotection, in case of traumatic brain injury (TBI), remains to be poorly understood. Clinical studies of cerebroprotectors - antagonists of NMDA-receptors indicate the feasibility of their use in practice. In our opinion, for further research, it would be correct to choose a compound that has neuroprotective properties and synthesized under the guidance of Academician M.O. Lozynsky at the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine, a derivative of 1-adamantylethyloxy-3-morpholoin-2-propanol hydroxide (Ademol). **Materials and methods.** The pharmacological action of Ademol was investigated on the example of a simulated TBI at a dose of 2 mg/kg intravenously every 12 hours for 8 days. The control group and pseudooperated animals received NaCl 0,9% at a dose of 2 mg/kg intravenously (i/v), the comparison group received Amantadine sulfate 5 mg/kg. To determine the efficiency and activity of these molecules the activity of the protein S 100 was used. **Results.** The dynamics of S 100 protein levels in groups of rats with Ademol and Amantadine sulfate with traumatic brain injury on the 8th day of observation indicates that on the background of the above therapy, the content of the studied marker decreased by an average of 50,8 and 39.9%. Treatment of rats with severe traumatic brain injury with Ademol at a dose of 2 mg/kg (i/v), was probably better than treatment of rats in the control group with saline and amantadine sulfate and better helped to reduce the growth of protein S 100 level, while Ademol exceeded the reference drug at 18,2% ($p < 0.05$).

Kew words: traumatic brain injury, ademol, amantadine sulfate, 0.9% NaCl, protein S 100

Introduction

Analyzing conducted research of the European Consensus on Brain Diseases, we understand that 700,000 people in the European Union suffer from the effects of traumatic brain injury (TBI) [10]. Forms of TBI that can lead to a large amount of fatal consequences and considered to be severe (concussion, compression of the brain by intracranial hematomas, diffuse axonal damage) are observed in 20-40% of patients with TBI [3]. Mortality of patients with TBI depends on the severity and nature of brain injury and ranges from 5 to 65% [14]. Speaking of disability in case of TBI, we understand that it is a long-term pathology, and in 30-35% of cases it is indefinite [9].

Continuous improvement and in-depth knowledge of pathophysiological brain damage requires the search for new pharmacological molecules that can facilitate the course and accelerate the recovery of patients after brain damage, which is also complicated by long and costly preclinical and clinical studies [13,14]. Excessive activation of NMDA receptors is observed in cases of brain injuries, which leads to acute excitotoxic neurodegeneration. In turn, the pathological reaction of glutamate-calcium damage joins, that is typical for strokes, which leads to secondary damage of brain structures [11]. Nowadays, there are no great and informative clinical investigations of neuroprotectors - NMDA receptor antagonists. Amantadine sulfate is a drug whose therapeutic effect is based on blocking the activity of NMDA-glutamate receptors, cholinoblocking action, blocking dopamine reuptake, enhancing the synthesis and release of dopamine into the synaptic cleft [6].

For investigation we have chosen promising molecule with neuroprotective properties and was synthesized under the guidance of Acad. M.O. Lozynsky at the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine, which is a derivative of adamantane 1adamantylethyloxy-3-morpholino-2-propanol

hydrochloride (laboratory code UK-1, conditional name Ademol). The protective effect of Ademol on the brain is associated with the effect on the activity of NMDA receptors, elimination of energy metabolism deficiency, metabolic acidosis, neuronal damage by free radicals, normalization of nitric oxide metabolism, normalization of blood flow in the brain, preservation of cortical cell structure. including, by means of apoptosis reducing [7]. This drug has an effect on a wide range of factors of brain damage, which is confirmed by studies [8].

Protein S 100, a biomarker of brain damage secreted by astrocytes [2, 11]. Protein S 100 regulates calcium homeostasis, which according to the pathogenesis of brain damage in trauma has neurotropic effects in the nanomolar range and neurotoxic effects in the micromolar range [4, 10]. Protein S 100 circulates from the cerebrospinal fluid into the blood and is excreted by kidneys. There is information about the significant amount of S 100 protein in leukocytes and the possibility of obtaining inflated levels of the studied indicator due to leukocyte lysis. Therefore, rapid analysis of S 100 protein will help to avoid erroneously high results [4].

The release of S 100 protein into the cerebrospinal fluid and blood is a marker of nerve tissue damage. The activity of S 100 protein in the blood of patients with TBI reflects the degree of brain damage [11].

According to the dynamics of S 100 protein activity in simulated brain pathology, we had a great interest in studying the effect of the drug on the activity of cell destruction processes in astrocytic glia in our model of brain pathology.

MATERIALS AND METHODS OF RESEARCH

To evaluate the influence effect of 1-adamantylethyloxy-3-morpholino-2-propanol hydrochloride (Ademol) comparing its activity with amantadine sulfate and 0,9% sodium chloride solution on the dynamics of S 100 protein activity in rats with simulated traumatic brain injury.

The study was performed on white male rats weighing 160-190 g, which were in standard vivarium conditions, in compliance with ethical standards for experimental studies in accordance with the "General Principles of Animal

Management", approved by the I National Congress of Bioethics (Kyiv, Ukraine, 2001) and the Law of Ukraine "On Protection of Animals from Cruelty" of February 26, 2006 [13]. The experimental model of TBI was caused by the action of carbon dioxide flow under pressure, created using a gas-balloon air pistol brand: "Baikal MP654K" (RF, Izhevsk, № certificate ROSS RU RU MZh03.V02518) and carbon dioxide cylinders with carbon dioxide 12 (Crosman, USA, № Series 456739). Rats under propofol anesthesia (60 mg/kg), after catheterization of the femoral vein and the possibility of infusion through the infusion machine, performed right-sided osteoplastic trepanation in the projection of the middle cerebral artery, hole diameter 5 mm^2. After fixing the rat, shot was done from a specific distance, a bone fragment on the periosteum together with the aponeurosis was returned to its place and the wound was sutured in layers. Thus, severe TBI was simulated. The therapeutic effect of Ademol (Ademol-Darnytsia, Darnytsia, Ukraine, 10 ampoules of 5 ml at a concentration of 1 mg / ml) on a model TBI was evaluated at a dose of 2mg/kg intravenously. Treatment was performed by slow intravenous infusion of infusomat, which lasted 2 times a day for 2 hours (every 12 hours) for 8 days. Treatment was started 1 hour after simulation of the pathological condition. Pseudooperated animals were subjected to all interventions (narcosis, skin incision, bone-plastic trepanation of the skull) except for manipulations that could directly lead to traumatic brain injury, which offset the impact of traumatic conditions of the experiment. They were also given an equivalent amount of 0,9% NaCl solution to a dose of Ademol. As drugs, for the control group, 0,9% NaCI solution at a dose of 2 ml/kg intravenous in the same mode was used, and for the comparison group - amantadine sulfate ("PC-Merz", Merz Pharmaceuticals, Switzerland, 200 mg/500 ml) on the model TBI was evaluated at a dose of 5 mg/kg intravenously in the same mode. The activity of S 100 protein in the blood of rats was measured on the 8th day of TBI by solid-phase enzyme-linked immunosorbent assay using the S 100 ELISA KIT on a device firm Hipson (Czech Republic).

The obtained data were processed on the basis of the statistical package "STATISTICA 6.1" (owned by the research centre of National Pirogov Memorial Medical University, licensed № VHXR901E246022FA). The results of protein levels S 100 are given as (M ± m), where M is the average value, m is the standard error. We used the Student's parametric test t for normal distribution, the Mann-Whitney U nonparametric test in its absence, and the Wilcoxon matched pairs test for significant changes in intra-group dynamics. The statistical significance of the difference between the comparative values was considered probable at $p<0,05$.

RESULTS AND DISCUSSION

From the data obtained at the end of our experiment on the 8th day of the model TBI analysis of protein S 100, shows a significant increase in the studied indicator in the control group probably an average of 7,49 times compared with the group of pseudooperated animals (Table 1).

Thus, the analysis of our data in the group TBI + saline according to the level of protein S 100 as a marker of nerve tissue damage allows us to conclude that on the 8th day of brain injury, in control rats group, intensive formation and organization of the alteration nucleus.

Table 1

The effect of course infusion of the studied cerebroprotectors on the dynamics of S 100 protein levels in rats with traumatic brain injury on the 8th day of experiment

(M±m, n=6)

Research groups	Protein content S 100 (ng / ml)
Pseudooperated animals + 0,9% solution NaCl	0,231±0,013

TBI + 0,9 % solution NaCl (controlled group)	1,73±0,07^{o} (+649%)
TBI + ademol, 2 mg/kg i/v	0,850±0,05$^{o*\#}$ (+268%),[-50,8%] {-18,2%}
TBI + amantadine sulfate, 5 mg/kg i/v	1,04±0,04^{o*} (350%),[-39,9%]

Notes:

1. TBI – traumatic brain injury;
2. ^{o} –p<0,05 relative to pseudooperated animals;
3. * – p<0,05 relative to the control pathology group;
4. $^{\#}$ – p<0,05 relative amantadine sultate
5. ( ) – relative to the rate of pseudooperated rats;
6. [ ] – relative to the rate of control pathology;
7. { }– regarding amantadine sulfate therapy.

The use of Ademol at a dose of 2 mg/kg as well as amantadine sulfate 5 mg/kg on the background of model TBI for 8 days reduced the level of the studied marker protein S 100, but at the end of the experiment its level remained significantly higher by 268% for the group TBI + Ademol and 350% for the TBI + amantadine sulfate group compared to the pseudooperative group.

S 100 protein is a specific astrocytic glia protein that is able to bind calcium. According to many researchers, at the time of injury, the activation of neuronal and glial metabolism starts, which leads to depletion of adenosine triphosphate (ATP), energy deficiency and depolarization of cell membranes [1]. Due to depolarization, potential-dependent calcium channels are activated and calcium ions from the extracellular space begin to enter the cell [5]. The consequence of calcium overload of the cell is its damage due to activation of phospholipases, proteases and nucleases, which leads to disruption of the integrity of the outer cell membrane and

mitochondrial membrane, interruption of oxidative phosphorylation, protein synthesis with cell expression. In patients with brain damage (e.g., traumatic brain injury, stroke), the level of protein S 100 may reflect the degree of brain damage [11].

Assessing the dynamics of S 100 protein levels in groups of rats with brain injury + Ademol (2 mg/kg) and brain injury + Amantadine sulfate (5 mg/kg), we found that on the background of the above therapy, the content of the studied marker decreased compared to with the control group on average 2,03 and 1,66 times, respectively, or 50,8 and 39,9%, respectively.

Thus, the data obtained in our study indicate that the use of Ademol at a dose of 2 mg/kg intravenous and amantadine sulfate at a dose of 5 mg/kg intravenous, against the background of model TBI in these drugs show strong neurocytoprotective properties. Moreover, this effect of Ademol was more pronounced, because the ability to inhibit the increase in protein levels of S 100, it probably outperformed the reference drug, respectively, by 18,2%.

CONCLUSIONS

1. Treatment of rats with severe traumatic brain injury with Ademol solution at a dose of 2 mg/kg intravenous, probably better than rats of the control pathology group with the introduction of saline and the group with amantadine sulfate reduces the increase in protein S 100, with Ademol predominating comparison drug by 18,2% ($p<0,05$).

2. The mechanism of brain protection in traumatic brain injury is the ability of Ademol to adjust the level of S 100 protein in severe cerebral trauma.

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