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PROTECTIVE EFFECT OF MAGNESIUM NITRATE ON BRAIN ISCHEMIA

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ABSTRACT

The influence of inorganic forms of magnesium: magnesium nitrate ($Mg(NO_3)_2$), magnesium sulfate ($MgSO_4$) and magnesium chloride ($MgCl_2$), administered in dose of 5mg/kg and 50mg/kg intraperitoneal for 1 hour before brain ischemia and 5 seconds after occlusion of common carotid arteries, on the course of ischemic stroke for 24 hours was studied. It was found that of all inorganic forms of magnesium only Mg $(NO_3)_2$ in dose of 5mg/kg and 50mg/kg had a significant protective effect on the dynamics of neurological disorders and mortality of animals for 24 hours.

KEYWORDS: Ischemic stroke, magnesium nitrate, nitric oxide.

INTRODUCTION

Identification of safe, effective, reasonably priced and easy-to-use medication for ischemic stroke (IS) treatment is considered of great importance. Such substances include drugs containing inorganic magnesium salts. Several international studies have shown that inorganic magnesium salts, i.e. magnesium sulfate ($MgSO_4$) and magnesium chloride ($MgCl_2$) are characterized by a neuroprotective effect.[1,2] Other studies have not provided evidence of the aforementioned effect.[3,4]

Recently, a powerful protective effect of magnesium nitrate (Mg $(NO_3)_2$) on the incomplete global cerebral ischemia has been shown.[5] It was also shown that $Mg(NO_3)_2$ lowers blood pressure.[6] The unique protective properties of this inorganic magnesium salt are manifested through the combined effect of its constituent ions: the magnesium cation (Mg^{2+}) and the nitrate anion (NO_3^-). The cation Mg^{2+} demonstrates a protective effect through neural and vascular mechanisms. Mg^{2+} is easily introduced and possesses a favorable safety profile,[7,8] it executes its protective effect by the early ATP stores regeneration in cells, inhibition of the neurotoxic glutamate release, calcium channels antagonism, inhibition of pro-inflammatory cytokines activation, etc.[9,10] The NO_3^- anion in the transformation chain ($NO_3^- \rightarrow NO_2^- \rightarrow NO$) is restored by nitrate/nitrite reductases.[11,12] to nitric oxide (NO), which mainly is responsible for the protective effect.[13] There is also evidence that NO_3^- anions can exert their protective properties through NO-independent mechanisms in consequence of the direct effect on key proteins and lipids.[14]

The purpose of this study is to compare the effects of magnesium inorganic forms, i.e. magnesium nitrate ($Mg(NO_3)_2$), magnesium sulfate ($MgSO_4$) and magnesium chloride ($MgCl_2$), with intraperitoneal administration of 5 mg/kg and 50 mg/kg doses 1 hour before cerebral ischemia and 5 seconds after the common carotid arteries occlusion, on the outcome of IS within 24 hours.

MATERIALS AND METHODS

To create a model of global cerebral ischemia, we used single-step ambilateral deligation of the common carotid arteries. Common carotid arteries in rats under ether anesthesia were released and ligated, the duration of intervention being no more than 10 minutes. After ether anesthesia, the rats quickly regained consciousness, animals were placed in separate cells and dynamics of neurological deficit was evaluated semi-quantitatively.[15] The assessment was made for restricted animal mobility, ptosis, hyperactive behavior, violent movements (rotations, jumps, convulsive and rotational seizures), limb paresis, coma and death. In accordance with the neurological symptoms assessment methodology applied, a state close to normal was marked as 0-3 points; 3-6 points marked intermediate severity; 7-24 points stood for severe degree of IS; the death of the animal was marked as 25 points. Neurological deficit was evaluated every 30 min for the duration of 24 hours. The total score for each time interval was averaged for all animals in the group. Based on the obtained data, graphs of the neurological disorders dynamics were constructed, with points put along the ordinate axis and time - on the abscissa axis.

448 Wistar rats weighing 120-140g were used in the experiments. All rats underwent occlusion of 2 carotid arteries. Four series of experiments were carried out, with 112 rats in each series. In the 1st and 3rd series of experiments drugs were injected 1 hour before cerebral ischemia, in the 2nd and 4th series – 5 seconds after common carotid arteries occlusion. In each series of the experiment, the animals were divided into 4 groups, three being experimental and one being a control group. There were 16 groups in total, 28 rats in each of them. The medication was administered in accordance with the following arrangement:

The first group (control), (n=28): the equivalent volume of normal saline (0.9% NaCl) was injected intraperitoneally at the same time intervals;

The second group (n=28): for each series the 5mg/kg and 50mg/kg doses of $MgSO_4$ were injected intraperitoneally 1 hour before cerebral ischemia and 5 seconds after the common carotid arteries occlusion.

The third group (n=28): for each series the 5mg/kg and 50mg/kg doses of $MgCl_2$ were injected intraperitoneally 1 hour before cerebral ischemia and 5 seconds after the common carotid arteries occlusion.

The fourth group (n=28): for each series the 5mg/kg and 50mg/kg doses of $Mg(NO_3)_2$ were injected intraperitoneally 1 hour before cerebral ischemia and 5 seconds after the common carotid arteries occlusion.

The significance of mean parameters difference in different experimental groups of animals was evaluated using the Mann-Whitney test (U Test) in the STATISTIKA 6 software. The variance ratio test Fisher (F test) was applied to assess the lethality of neurological manifestations.

RESULT

The influence of 5 mg/kg doses of Mg $(NO_3)_2$, $MgSO_4$ и $MgCl_2$ administered 1 hour before the cerebral ischemia on IS course.

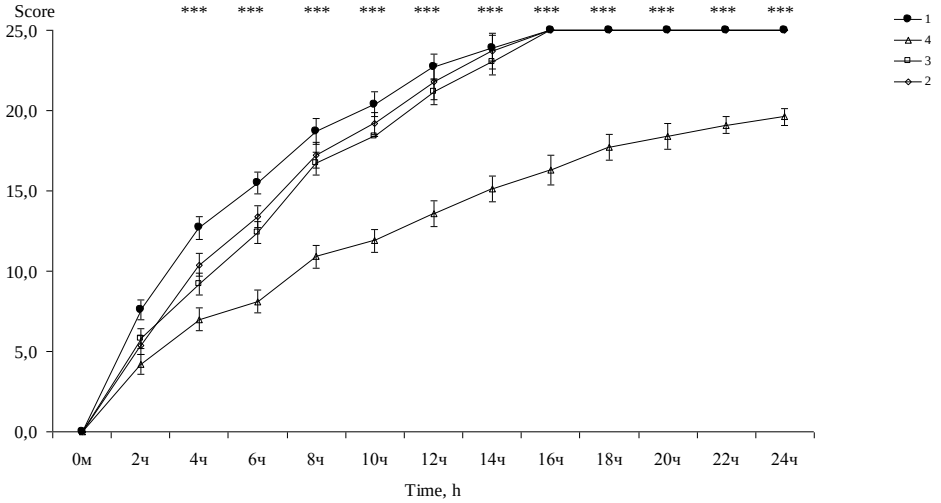

Fig. 1: The influence of $Mg(NO_3)_2$, $MgSO_4$ и $MgCl_2$ administered 1 hour before the cerebral ischemia on IS course.

1-control; 2- $MgSO_4$ in 5 mg/kg dose; 3- $MgCl_2$ in 5 mg/kg dose; 4 – Mg $(NO_3)_2$ in 5 mg/kg dose. *** $p<0,001$ – significance of differences between 4^{th} и 1^{st}; 4^{th} и 2^{nd}; 4^{th} и 3^{rd} groups

The neurological disorders increase intensity in the group of rats with 5 mg/kg dose of Mg $(NO_3)_2$ injected 1 hour before ischemia, during 24 hours starting from the 240th minute was significantly ($p < 0.001$) lower than in the control group of animals and then in the groups that were administered $MgSO_4$ and $MgCl_2$ at a dose of 5 mg/kg (Fig. 1). The death rate in group 4 was significantly ($p < 0.001$) lower than in groups 1, 2 and 3. No significant differences between groups 1, 2 and 3 were observed (Fig. 1).

The influence of 5 mg/kg doses of Mg $(NO_3)_2$, $MgSO_4$ и $MgCl_2$ administered 5 seconds after the cerebral ischemia on IS course.

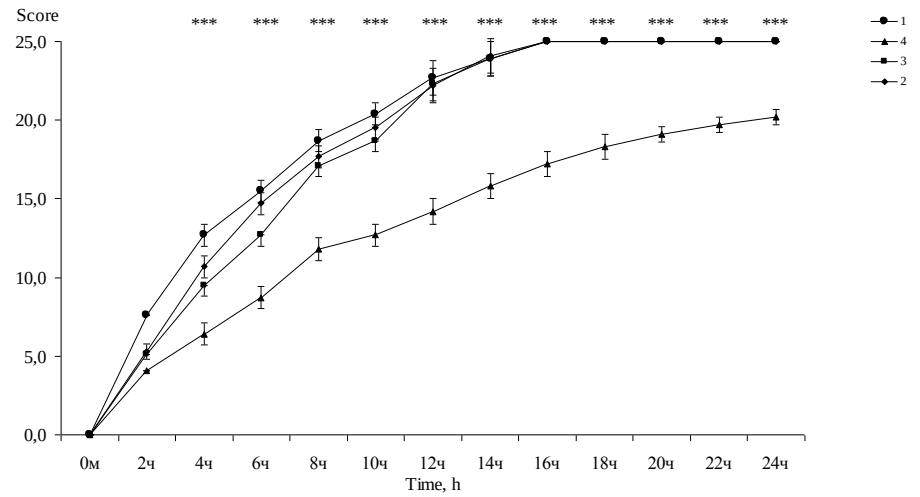

Fig. 2: The influence of $Mg(NO_3)_2$, $MgSO_4$ и $MgCl_2$ administered 5 seconds after the cerebral ischemia on IS course.

1-control; 2- $MgSO_4$ in 5 mg/kg dose; 3- $MgCl_2$ in 5 mg/kg dose; 4 - $Mg(NO_3)_2$ in 5 mg/kg dose. *** p<0,001 – significance of differences between 4th и 1st; 4th и 2nd; 4th и 3rd groups

In animals that were injected with $Mg(NO_3)_2$ 5 mg/kg doses in 5 seconds after two carotid arteries occlusion, the neurological deficit was significantly less prominent (p <0.001) from the 240th minute and until the end of the experiment, than in the 1st, 2nd and 3rd groups of rats (Fig. 2). The death rate in group with $Mg(NO_3)_2$ injected in 5 mg/kg doses was significantly (p <0.001) lower than in groups 1, 2 and 3. No significant differences between groups 1, 2 and 3 were observed (Fig. 2).

The influence of 50 mg/kg doses of Mg $(NO_3)_2$, $MgSO_4$ и $MgCl_2$ administered 1 hour before the cerebral ischemia on IS course.

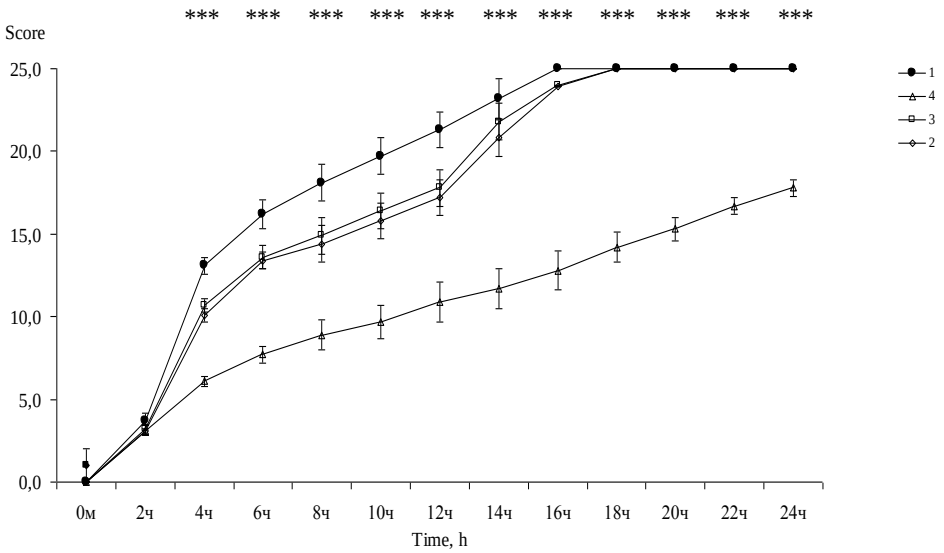

Fig. 3: The influence of $Mg(NO_3)_2$, $MgSO_4$ и $MgCl_2$ administered 1 hour before the cerebral ischemia on IS course.

1-control; 2- $MgSO_4$ in 50 mg/kg dose; 3- $MgCl_2$ in 50 mg/kg dose; 4 - $Mg(NO_3)_2$ in 50 mg/kg dose. *** p<0,001 – significance of differences between 4th и 1st; 4th и 2nd; 4th и 3rd groups.

The neurological symptoms increase intensity in group 4 for 24 hours was significantly lower (p <0.001) than in groups 1, 2 and 3 (Fig. 3). The death rate in group 4 was significanty (p<0,001) lower than in groups 1, 2 and 3. No significant differences between groups 1, 2 and 3 were observed (Fig. 3).

The influence of 50 mg/kg doses of $Mg(NO_3)_2$, $MgSO_4$ и $MgCl_2$ administered 5 seconds after the cerebral ischemia on IS course.

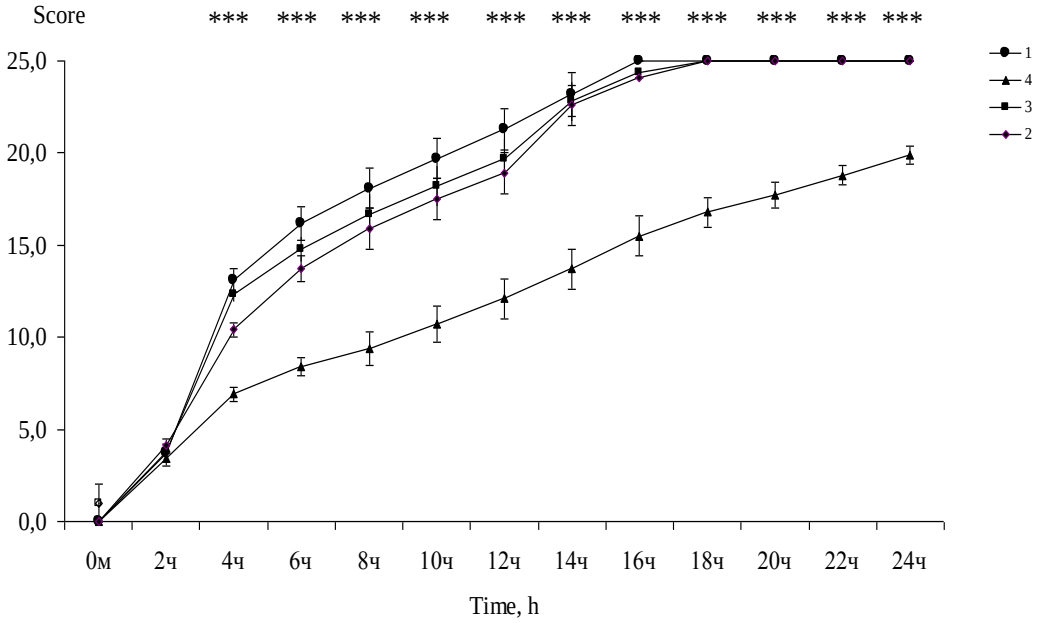

Fig. 4: The influence of $Mg(NO_3)_2$, $MgSO_4$ и $MgCl_2$ administered 5 seconds after the cerebral ischemia on IS course.

1-control; 2- $MgSO_4$ in 50 mg/kg dose; 3- $MgCl_2$ in 50 mg/kg dose; 4 - $Mg(NO_3)_2$ in 50 mg/kg dose. *** $p<0,001$ – significance of differences between 4^{th} и 1^{st}; 4^{th} и 2^{nd}; 4^{th} и 3^{rd} groups

In rats administered with 50 mg/kg doses of $Mg(NO_3)_2$ 5 seconds after cerebral ischemia, the neurological deficiency within 24 hours was significantly ($p <0.001$) less pronounced than in group 4 (Fig. 4). The death rate in group 4 was significantly lower ($p <0.001$) than in the control group. In rats administered with 50 mg/kg doses of $MgSO_4$ and $MgCl_2$ 5 seconds after cerebral ischemia, the neurological disorders increase intensity within 24 hours was significantly ($p <0.001$) higher than in rats administered with 50 mg/kg doses of $Mg(NO_3)_2$ (Fig.4). The death rate in group 4 was significantly ($p<0,001$) lower as well than in groups 2 and 3. No significant differences between groups 1, 2 and 3 were observed (Fig. 4).

Despite the fact that there were no significant differences between groups 1, 2 and 3, the neurological disorders increase intensity in groups 2 and 3 was less intense than in the control group of rats (Fig. 1-4).

DISCUSSION

Injection of 5mg/kg and 50 mg/kg doses of $Mg(NO_3)_2$ significantly ($p<0,001$) decreased neurological disorders caused by two carotid arteries occlusion (Fig. 1-4). Such a powerful protective effect of $Mg(NO_3)_2$ is probably associated with synergistic effect of Mg^{2+} cation and NO_3^- anion constituent of $Mg(NO_3)_2$. This may be due to three factors: (1) the transformation of nitrates into NO by nitrate/nitrite-reductase enzyme system, (2) the action of the Mg^{2+} cations, and (3) the non-toxicity of the NO_3^- anions introduced in physiological concentration, compared to toxic anions SO_4^{2-} and Cl^-, introduced in the same doses.

(1) It is known that during hypoxia/cerebral ischemia, the increase is observed in the enzymatic activity of nitrate/nitrite-reductases, which carry out a sequential chain of transformation $NO_3^- \rightarrow NO_2^- \rightarrow NO$.[11,12] A moderate increase in NO concentration, which is a powerful vasodilator, leads to thrombocyte aggregation inhibition, increased blood flow in the brain, etc., which leads to a neurological deficit decrease.[13] The NO_3^- anions can also exert their protective effects through the NO-independent mechanism in consequence of their direct action on key proteins and lipids.[14]

(2) The activity of Mg_2^+ cations reduces thrombocyte aggregation, blocks calcium channels, reduces excessive entry of Ca^{2+} ions into cells, inhibits NMDA receptors, thus reducing excitotoxicity, promotes early recovery of cellular ATP stocks, which reduces the damaging effect of ischemia/hypoxia on cerebral tissue.[9]

(3) The insignificant unreliable protective effect that was observed with the administration of 5 mg/kg and 50 mg/kg doses of $MgSO_4$ and $MgCl_2$, is probably associated with Mg^{2+} cations (Fig. 1-4). The inorganic magnesium agent $Mg(NO_3)_2$ also has a Mg_{2+} cation, but $Mg(NO_3)_2$ had a significant ($p <0.001$) protective effect on cerebral ischemia compared to $MgSO_4$ and $MgCl_2$. Apparently, such a significant difference between inorganic magnesium salts is associated with magnesium counter-ions, namely the anions NO_3^-, SO_4^{2-} and Cl^-. Numerous studies demonstrate that SO_4^{2-} and Cl^- anions manifest toxic properties.[16] Probably, the failures in medical and experimental practice associated with the

use of $MgSO_4$ and $MgCl_2$ salts, are related to magnesium counter anions, namely SO_4^{2-} and Cl^-.

The experiment results showed that the protective effect of $Mg(NO_3)_2$ did not depend on the time of administration. As seen from Fig.1-4, the introduction of $Mg(NO_3)_2$ both 60 minutes before the carotid arteries occlusion, and 5 seconds after the development of ischemia, had a significant ($p < 0.001$) protective effect. Although, it should be noted that the early introduction of inorganic magnesium agents led to a less intensive increase in neurological symptoms (Fig. 1-4).

Thus, a significant protective effect of $Mg(NO_3)_2$ in doses of 5 mg/kg and 50 mg/kg on incomplete cerebral ischemia is associated with the synergistic effect of Mg^{2+} cations and NO_3^- anions. Presumably the ongoing experimental and medical research into the therapeutic effects of inorganic magnesium medication will be associated with studying the effects of Mg $(NO_3)_2$. Magnesium nitrate meets many of the requirements for ideal pharmacological agents: inexpensive, affordable, easily administered, and apparently without adverse side effects.

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DISCLOSURE

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