DOI 10.1007/s10517-014-2651-5
Bulletin of Experimental Biology and Medicine, Vol. 157, No. 6, October, 2014 GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Protective Effect of Magnesium Nitrate against Neurological Disorders Provoked by Cerebral Ischemia in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 157, No. 6, pp. 688-691, July, 2014
Original article submitted June 24, 2013

The study examined effects of inorganic magnesium agents: magnesium nitrate $Mg(NO_3)_2$, magnesium sulfate $MgSO_4$, and magnesium chloride $MgCl_2$ on the development of neurological disorders and mortality in rats resulting from cerebral ischemia provoked by a single-stage bilateral occlusion of the common carotid arteries. The rats were injected with one of examined magnesium preparations (5 mg/1 kg body weight) 1 h prior to or 1-2 sec after occlusion. The control group rats were treated with physiological saline at the same terms. Irrespective of the moment of injection, magnesium nitrate demonstrated significant protective effect on dynamics of neurological disorders and mortality, while similar effects of magnesium sulfate and magnesium chloride were insignificant.

Key Words: *neurological disorders; magnesium nitrate*; *nitric oxide*

For several decades, magnesium and nitrates are used as the protective drugs in treatment of cardiovascular diseases, including stroke [8,12]. It is common knowledge that ischemic brain disease is characterized by a complex cascade of pathobiochemical reactions in the brain tissue. Each of these reactions includes numerous metabolic pathways. Routine drugs used in the therapy of stroke are aimed at selective modification of the metabolic pathway at a certain stage in the pathological process. This approach is often little effective, so the researchers focus on the search for protective drugs with pleiotropic effects [1]. These drugs should be effective, reasonably priced, and easily available. Among promising protective and pleiotropic drugs, one can consider magnesium nitrate $Mg(NO_3)_2$. Since this agent contains both magnesium cation Mg^{2+} and nitrate anion NO_3^-, it should demonstrate the protective properties of the inorganic preparations of magnesium and nitrate.

In recent decade, medical and scientific community radically revised the views on deleterious effects of nitrates. According to modern views, nitrates are not related with the development of methemoglobinemia and cancerogenesis. They produce selective therapeutic effects on the cardiovascular system via the formation of NO or employing the NO-independent mechanism based on direct action on the key proteins and lipids [6,11]. Under conditions of oxygen deficiency, many enzymes modify their function. For instance, during hypoxia/ischemia, metal-containing enzymes such as deoxyhemoglobin, deoxymyoglobin, NO-synthase, *etc.*, play the role of nitrate/nitrite reductases. Their substrates, nitrates (NO_3^-) and nitrites (NO_2^-) become alternative sources for NO generation [3,9,13]. Conversion of the nitrates into NO under conditions of hypoxia/ischemia is an ancient protective mechanism, which lost this role during evolution; otherwise, NO-synthase is erroneously considered as the major NO-synthesizing enzyme.

Magnesium cation Mg^{2+} is the second (after calcium) intracellular regulatory cation acting as a co-factor in more than 500 proteins participating in numerous biochemical reactions [10]. Magnesium exerts its neuroprotective effects via antagonism with calcium ions in calcium channels, non-competitive antagonism at NMDA receptors, inhibition of release of neurotoxic glutamate, down-regulation of oxygen radicals generation and excessive production of NO, as well as early restoration of ATP reserves in the cells [7,14].

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Our aim was to examine the effect of inorganic magnesium forms, *i.e.*, magnesium nitrate $Mg(NO_3)_2$, magnesium sulfate $MgSO_4$, and magnesium chloride $MgCl_2$ on neurological disorders provoked by carotid occlusion in rats.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 120-150 g. The global cerebral ischemia was modeled by single-stage ligation of both carotid arteries [5]. Under ether narcosis, the common carotid arteries were exposed and ligated (the procedure took <10 min; the duration of ischemia 8 h). After ether narcosis, the rats rapidly woke up and were placed into individual cages, where the course of neurological symptoms [4] was assessed semiquantitatively by the decrease in motor activity, ptosis, hyperactive behavior, compulsive movements (rotations, jumps, as well as compulsive and rotational paroxysms), paresis of limbs, coma, and death. The 25-point scale was used to score the quasinormal state (0-3), the mean degree of ischemic stroke (3-6), severe degree of ischemic stroke (7-24), and the death (25). The neurological symptoms were assessed every 30 min over 8 h. In each group, the score was averaged for every observation interval. The development of neurological disorders was shown in the point-time plots.

Two series of experiments were carried out on n=112 rats in each series. In all rats, both common carotid arteries were ligated. In series I, the agents were intraperitoneally injected 1 h prior to cerebral ischemia, while in series II, they were injected in 1-2 sec after ligation of the arteries. In each series, the rats were randomized into one control and 3 experimental groups. Each group comprised 28 rats.

Controls (group 1) received injections of 0.9% NaCl. The experimental group rats received the examined agent (5 mg/kg): $MgSO_4$ (group 2 in each series), $MgCl_2$ (group 3), or $Mg(NO_3)_2$ (group 4).

The data were analyzed statistically using Statistica 6.0 software. Significance was assessed by the non-parametric Mann–Whitney U test. Significance of mortality was calculated by Fisher's test. Significant differences in animal mortality were observed starting from minute 330 after ligation.

RESULTS

During postligation minutes 390-480, the experimental group 4 rats treated with $Mg(NO_3)_2$ 1 h prior to ligation (series I) demonstrated significantly milder (p<0.05) neurological disorders than the rats in control and other experimental groups in this series (Fig. 1). Mortality in $Mg(NO_3)_2$ group (15.7%) was significantly lower (p<0.05) than in the control (42.0%), $MgSO_4$ (30.1%), and $MgCl_2$ (35.4%) groups. In this series, there were no significant differences in the course of ischemic insult between the control rats and the rats treated with $MgSO_4$ or $MgCl_2$ (Fig. 1).

In rats, treated with $Mg(NO_3)_2$ 1-2 sec after ligation of carotid arteries (series II), the neurological symptoms observed on postligation minutes 360-480 were significantly (p<0.05) less pronounced than in the control and two other experimental groups (Fig. 2). Mortality in $Mg(NO_3)_2$ group (5.9%) was significantly smaller (p<0.05) than that in control (42.0%), $MgSO_4$ (33.3%), and $MgCl_2$ (35.0%) groups. In this series, there were no significant difference in the course of ischemic insult between the control rats and the rats treated with $MgSO_4$ or and $MgCl_2$ (Fig. 2).

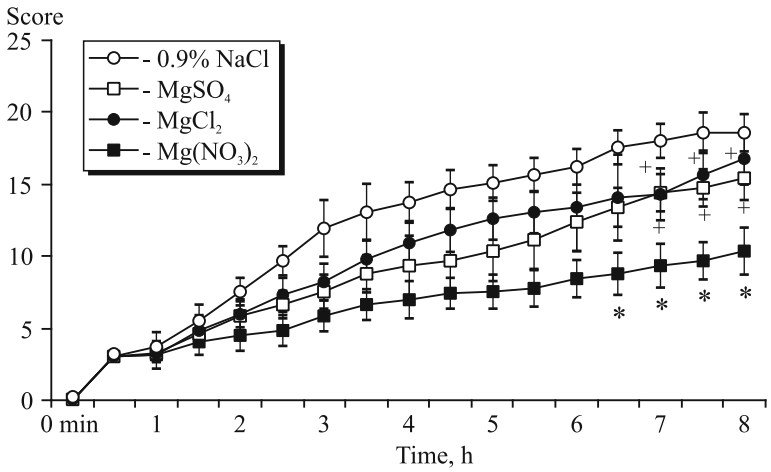

Fig. 1. Effect of inorganic magnesium preparations injected 1 h prior to cerebral ischemia on the course of ischemic stroke. Here and in Fig. 2: p<0.05 relative to *0.9% NaCl, $^+MgCl_2$ and $MgSO_4$.

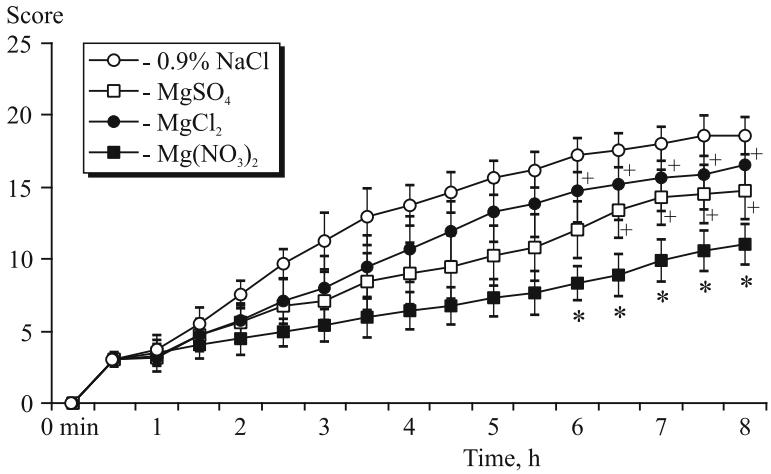

Fig. 2. Effect of inorganic magnesium preparations injected 1-2 sec after cerebral ischemia on the course of ischemic stroke.

Thus, the protective effect of $Mg(NO_3)_2$ did not depend on the time of injection (Figs. 1, 2).

Probably, the significant protective effect of $Mg(NO_3)_2$ against incomplete total cerebral ischemia resulted from the combined action of Mg^{2+} and NO_3^- ions. The reasons are twofold. First, $Mg(NO_3)_2$ is a donor of NO [8]. The latter is known to up-regulate cerebral circulation, down-regulate aggregation of the platelets, and inhibit adhesion of leukocytes. Moderate elevation of NO concentration induces transition of proteins from soluble to membrane-bound state [3], which can strengthen stability of both proteins and membrane. Second, Mg^{2+} ions inhibit adhesion of leukocytes and aggregation of the platelets. These ions block calcium channels, prevent excessive entry of calcium ions into the cells, inhibit NMDA receptors thereby moderating excitotoxicity, and promote the early restoration of cellular ATP stock [1,7,12]. The following fact seems also indicate the combined effect of Mg^{2+} and NO_3^- ions on incomplete cerebral ischemia. It is a common knowledge that the nitrate/nitrite reducing capacity of Na^+ cation is greater than that of Mg^{2+} cation, so production of NO from sodium nitrate ($NaNO_3$) is higher than that from magnesium nitrate which means that $NaNO_3$ is a more potent vasodilator than $Mg(NO_3)_2$. However, when applied in equal dose of 5 mg/kg, only $Mg(NO_3)_2$ demonstrated significant protective effect against cerebral ischemia [2].

Thus, the data obtained suggest that the protective effect of $Mg(NO_3)_2$ in the dose of 5 mg/kg against incomplete cerebral ischemia results from the combined effect of Mg^{2+} cations and NO_3^- anions. Probably, the anions $(SO_4)^{2-}$ and Cl^- are responsible for unsuccessful use of $MgSO_4$ and $MgCl_2$ in medical practice. Evidently, the effective, inexpensive, and easily available magnesium nitrate $Mg(NO_3)_2$ holds much promise for prevention and therapy of cerebral ischemia.

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