ISSN 0096-3925, Moscow University Biological Sciences Bulletin, 2014, Vol. 69, No. 4, pp. 147–152. © Allerton Press, Inc., 2014.
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PHYSIOLOGY

Protective Effect of Magnesium Nitrate on Brain Ischemia

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Received April 13, 2013

Abstract—Effects of magnesium nitrate ($Mg(NO_3)_2$) on the dynamics of neurological disorders and the lethality rate after brain ischemia induced by simultaneous bilateral occlusion of common carotid arteries were investigated in rats. $Mg(NO_3)_2$ administered in doses of 5 mg/1000 g and 50 mg/1000 g 60 min before and 1–2 s after the occlusion of both common carotid arteries statistically reduced the severity of neurological impairment and the death rate of rats.

Keywords: brain ischemia, magnesium nitrate, nitric oxide.

DOI: 10.3103/S0096392514040075

INTRODUCTION

In recent decades, there appeared a vast number of scientific works suggesting that nitrates and nitrites are not only harmless, but can also have some therapeutic potential, especially in the case of cerebral vascular disorders [1–2]. It was determined, as early as in the beginning of the 20th century that, under a dietary regimen excluding nitrates, nitrites (NO_2^-) and nitrates (NO_3^-) were still present in the organism [3]. It was observed that the rate of cardiovascular diseases is quite low in the countries where large quantities of fruits and vegetables containing nitrates and nitrites are consumed [4, 5]. This is why scientists turned their attention to the possibility of using nitrates for the treatment of ischemic brain pathologies that are widespread and have bad after-effects for human health [6]. It was also determined that nitrates and nitrites are not just the inert products of nitric oxide (NO) metabolism but rather the form of NO storage [7]. It was discovered that nitrates and nitrites exert their positive effects on the vascular system of ischemic brain through NO generation [8]. It is well known that, under the conditions of oxygen deficiency or ischemia, nitrate and nitrite reductases catalyze the following chain of molecular conversions, $NO_3^- \longrightarrow NO_2^- \longrightarrow NO$ [9]. Moreover, under the oxygen shortage conditions, when NO synthase (NOS) enzyme is not able to synthesize NO, many other enzymes change their mode of action. For instance, during the period of ischemia or hypoxia, deoxyhemoglobine and NOS act as nitrite reductases [10, 11]. Thus, under oxygen shortage conditions, alternative ways of NO production become active, and nitrates and nitrites are substrates for the corresponding enzymes. This may appear to be some ancient mechanism of the organism protection from ischemia or hypoxia that has lost its leading role with time, or, alternatively, NOS is erroneously regarded to be the major NO synthesizing enzyme. There also exists data that nitrates and nitrites can realize their protective action by NO-independent mechanism that consists in directly affecting the key proteins and lipids [12].

Among other agents used in ischemic stroke treatment, inorganic magnesium compounds (magnesium sulfate and magnesium chloride) can be mentioned, which showed neuroprotective effects in a number of studies [13, 14]. Magnesium is the fourth most abundant cation in the human body, the second most abundant intracellular cation, and is a cofactor for more than 500 proteins involved in various biochemical reactions and signaling cascades [15, 16]. It is assumed that magnesium executes its neuroprotective action through the early regeneration of cellular ATP resources, antagonism of all types of calcium channels, noncompetitive antagonism of NMDA receptors, inhibition of neurotoxic glutamate release, inhibition of oxygen radicals generation and excessive nitrogen oxides formation, inhibition of proinflammatory cytokine activation, etc. [17, 18]. In the course of experimental and clinical studies, it was observed that administration of inorganic magnesium compounds mitigates negative effects of ischemic cascade [19]. In other studies, no such effects were detected [20, 21]. Proceeding from the position that both nitrates and inorganic magnesium compounds exert neuroprotective effects on the brain vascular system, we decided to test the joint effect of these two ions in incomplete brain ischemia.

The goal of the current work was to study the effects of inorganic forms of magnesium, namely magnesium nitrate ($Mg(NO_3)_2$), magnesium sulfate ($MgSO_4$), and

magnesium chloride ($MgCl_2$), on the progression of ischemic stroke when administered intraperitoneally in the doses of 5 mg per kg and 50 mg per kg 1 hour prior to brain ischemia and 1–2 s after the occlusion of common carotid arteries.

MATERIALS AND METHODS

In order to model the global brain ischemia, simultaneous bilateral occlusion of common carotid arteries was performed. In the rats under ether anesthesia, common carotid arteries were externalized and a ligation was placed around. The operation lasted for no more than 10 min. Rats quickly recovered after the ether anesthesia. Animals were put into separate cages, and the dynamics of neurological deficit was assessed semiquantitatively [23]. The following features were assessed: restriction of mobility, ptosis, hyperactive behavior, involuntary movements (rotations, jumps, convulsive attacks, and rotational attacks), extremities pareses, coma, and death. According to the neurologic symptoms assessment approach used, animal state close to normal was given 0–3 points, animals with the medium severity of pathological symptoms were given 3–6 points, animals with severe ischemic stroke were given 7–24 points, and animal death was made equal to 25 points. Neurological deficit was assessed every 30 min during the eight hour period. Total number of points (score) obtained in each time interval was made average for all the animals in the group. On the basis of the obtained data, neurological impairment dynamics plots were constructed, with score as the Y axis and time period as the X axis.

For the experiment, we used 448 Wistar strain rats with weight varying from 120 to 140 g. In all rats, the occlusion of both common carotid arteries was performed. Four series of experiments were carried out; we used 112 rats in each series. In the first and the third series, analyzed compounds were administered 1 hour prior to brain ischemia; in the second and the forth series, analyzed compounds were administered 1–2 s after the occlusion of common carotid arteries. In each series, all animals were subdivided into four groups, three of them being experimental groups and the fourth being the control. There were 16 groups in total, each containing 28 rats. The medications were administered according to the following scheme:

To *the first group*—control group ($n = 28$)—equal volume of physiological solution (0.9% NaCl) was administered intraperitoneally at the same time before and after the occlusion as in the experimental groups.

To *the second group* in each series ($n = 28$), $MgSO_4$ in the dose of 5 mg/kg or 50 mg/kg was administered intraperitoneally 1–2 s after the occlusion of common carotid arteries.

To *the third group* in each series ($n = 28$), $MgCl_2$ was administered intraperitoneally in the doses of 5 mg/kg or 50 mg/kg 1 hour prior to the brain ischemia or 1–2 after the occlusion of common carotid arteries.

To *the forth group* in each series ($n = 28$), $Mg(NO_3)_2$ was administered intraperitoneally in the doses of 5 mg/kg or 50 mg/kg 1 h prior to the brain ischemia and 1–2 s after the occlusion of common carotid arteries.

Statistical significance of differences in the average parameter values between the experimental groups of animals was assessed by means of the Mann–Whitney criterion (U Test) implemented in Statistica 6 software. Fisher criterion was used to assess the lethality of neurological symptoms.

RESULTS AND DISCUSSION

Effects of $Mg(NO_3)_2$, $MgSO_4$, and $MgCl_2$ Administration in the Dose of 5 mg/kg 1 Hour before Brain Ischemia on the Progress of Ischemic Stroke

The rate of neurological impairment progression during the period of 300–480 min of the experiment was statistically lower ($p < 0.01$) in the group of rats to which $Mg(NO_3)_2$ was administered in the dose of 5 mg/kg 1 h prior to the occlusion of both common carotid arteries than in the animals from the control group (Fig. 1). Animal lethality was also statistically lower ($p < 0.05$) in group 4 than in the control group. Neurological deficit was statistically less severe ($p < 0.05$) in group 4 to which $Mg(NO_3)_2$ was administered than in groups where $MgSO_4$ and $MgCl_2$ was injected in the dose of 5 mg/kg (Fig. 1). The lethality rate was statistically lower ($p < 0.05$) in group 4 than in groups 2 and 3. No statistically significant differences between groups 1 and 2 and between groups 1 and 3 were observed (Fig. 1).

Effects of $Mg(NO_3)_2$, $MgSO_4$, and $MgCl_2$ Administration in the Dose of 5 mg/kg 1–2 s after Brain Ischemia on the Progress of Ischemic Stroke

In the animals to which $Mg(NO_3)_2$ was administered in the dose of 5 mg/kg 1–2 s after the occlusion of both common carotid arteries, neurological deficit during 360–480 min of the experiment was significantly ($p < 0.05$) less evident than in the rats from the control group (Fig. 2). The lethality rate in the group of rats where $Mg(NO_3)_2$ was administered in the dose of 5 mg/kg was statistically lower ($p < 0.05$) than in the control group of animals. The rate of neurological impairment progression during 360–480 min of the experiment was statistically higher ($p < 0.05$) in the rats to which $MgSO_4$ and $MgCl_2$ were administered in the dose of 5 mg/kg 1–2 s after brain ischemia than in the rats to which $Mg(NO_3)_2$ was administered in the same dose (Fig. 2). Lethality was statistically lower in group 4 ($p < 0.05$) than in groups 2 and 3. No statistically significant differences between groups 1 and 2 and between groups 1 and 3 were observed (Fig. 2).

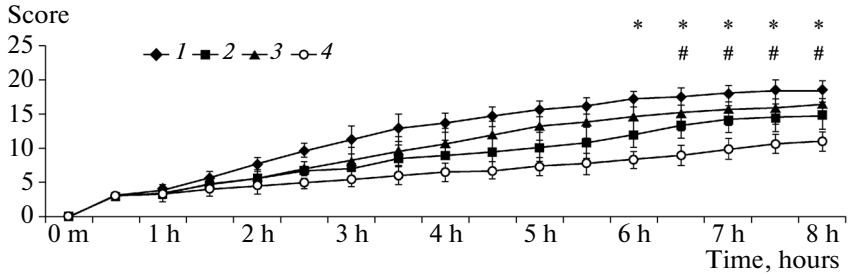

Fig. 1. Effects of $Mg(NO_3)_2$, $MgSO_4$, and $MgCl_2$ administration 1 h prior to brain ischemia on ischemic stroke progression. *1*—control; *2*—$MgSO_4$ in the dose of 5 mg/kg; *3*—$MgCl_2$ in the dose of 5 mg/kg; *4*—$Mg(NO_3)_2$ in the dose of 5 mg/kg. $^{**}p < 0.01$—statistical significance of the differences between the fourth and the first groups; $\#p < 0.05$—statistical significance of the differences between the second, the third, and the fourth groups.

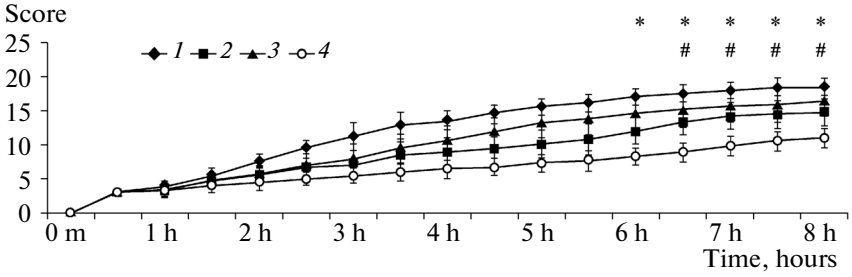

Fig. 2. Effects of $Mg(NO_3)_2$, $MgSO_4$, and $MgCl_2$ administration 1–2 s after brain ischemia on ischemic stroke progression. *1*—control; *2*—$MgSO_4$ in the dose of 5 mg/kg; *3*—$MgCl_2$ in the dose of 5 mg/kg; *4*—$Mg(NO_3)_2$ in the dose of 5 mg/kg. $^{**}p < 0.01$—statistical significance of the differences between the fourth and the first groups; $\#p < 0.05$—statistical significance of the differences between the second, the third, and the fourth groups.

Effects of $Mg(NO_3)_2$, $MgSO_4$, and $MgCl_2$ Administration in the Dose of 50 mg/kg 1 Hour before Brain Ischemia on the Progress of Ischemic Stroke

Administration of $Mg(NO_3)_2$ in the dose of 50 mg/kg 1 h before the occlusion of carotid arteries had pronounced protective effect, which revealed itself in less intensive progression of severe neurological symptoms and lower lethality rate in the corresponding experimental group in comparison with the control group of animals. Neurological deficit progression rate during the period of 210–480 min of the experiment appeared to be statistically lower ($p < 0.01$) in the corresponding experimental group than in the control group (Fig. 3). Lethality in the experimental group of rats was statistically lower ($p < 0.01$) than in the control group as well. Neurological symptoms during the time period from the 399th until the 480th min of the experiment were statistically less evident in the rats to which $Mg(NO_3)_2$ was administered in the dose of 50 mg/kg 1 h before brain ischemia compared to the animals to which $MgSO_4$ in the dose of 50 mg/kg (group 2) or $MgCl_2$ in the dose of 50 mg/kg (group 3) was administered prior to brain ischemia (Fig. 3). The lethality rate also proved to be lower ($p < 0.05$) in the fourth group than in the second and the third groups. No statistical differences were observed between groups 1 and 2 and between groups 1 and 3, though the neurological deficit progression rate was lower in groups 2 and 3 than in the control group (Fig. 3).

Effects of $Mg(NO_3)_2$, $MgSO_4$, and $MgCl_2$ Administration in the Dose of 50 mg/kg 1–2 s after Brain Ischemia on the Progress of Ischemic Stroke

In the rats to which $Mg(NO_3)_2$ was injected in the dose of 50 mg/kg 1–2 s after brain ischemia, the neurological deficit during the time period between 240 and 480 min of the experiment was statistically less evident ($p < 0.01$) than in the rats from the control group (Fig. 4). Lethality was also statistically lower in group 4 ($p < 0.05$) than in the control group. The rate of neurological impairment progression was statistically higher ($p < 0.05$) in the rats to which $MgSO_4$ or $MgCl_2$ was administered in the dose of 50 mg/kg 1–2 s after brain ischemia than in the rats to which $Mg(NO_3)_2$ was injected in the dose of 50 mg/kg (Fig. 4). The lethality rate in group 4 was also statistically ($p < 0.05$) lower than in groups 2 and 3. No statis-

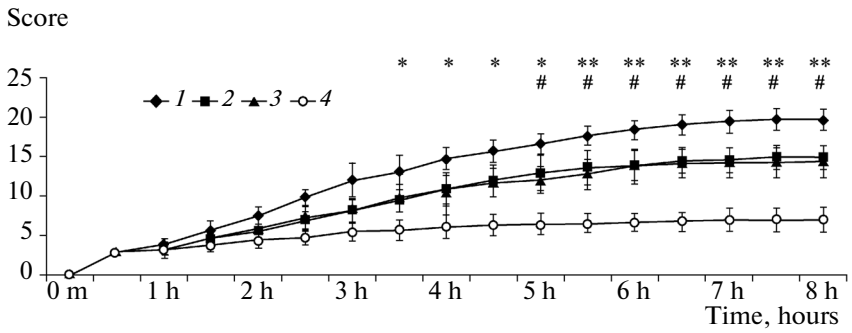

Fig. 3. Effects of $Mg(NO_3)_2$, $MgSO_4$, and $MgCl_2$ administration 1 h prior to brain ischemia on ischemic stroke progression. *1*—control; *2*—$MgSO_4$ in the dose of 50 mg/kg; *3*—$MgCl_2$ in the dose of 50 mg/kg; *4*—$Mg(NO_3)_2$ in the dose of 50 mg/kg. *$p < 0.05$—statistical significance of the differences between the fourth and the first groups; **$p < 0.01$—statistical significance of the differences between the fourth and the first groups; #$p < 0.05$—statistical significance of the differences between the second, the third, and the fourth groups.

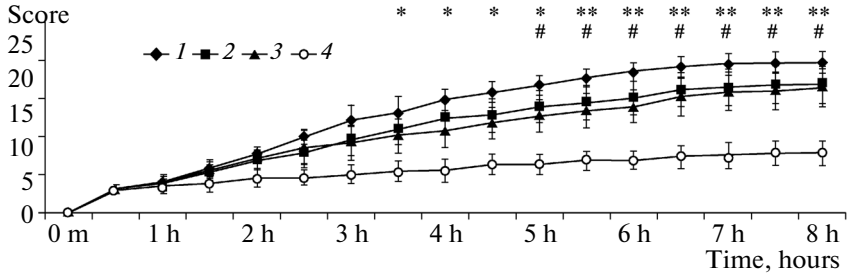

Fig. 4. Effects of $Mg(NO_3)_2$, $MgSO_4$, and $MgCl_2$ administration 1–2 s after brain ischemia on ischemic stroke progression. *1*—control; *2*—$MgSO_4$ in the dose of 50 mg/kg; *3*—$MgCl_2$ in the dose of 50 mg/kg; *4*—$Mg(NO_3)_2$ in the dose of 50 mg/kg. *$p < 0.05$—statistical significance of the differences between the fourth and the first groups; **$p < 0.01$—statistical significance of the differences between the fourth and the first groups; #$p < 0.05$—statistical significance of the differences between the second, the third, and the fourth groups.

tical differences were observed between groups 1 and 2 and between groups 1 and 3 (Fig. 4).

DISCUSSION

In the course of various clinical and experimental studies, it was determined that treatment with nitrates and magnesium compounds demonstrated protective effects for cerebral ischemia [1, 13, 14]. As long as $Mg(NO_3)_2$ contains both Mg^{2+} cation and NO_3^- anion, it can be assumed that it should combine the protective properties of both inorganic magnesium compounds and nitrates.

In our study, indeed, the administration of $Mg(NO_3)_2$ in the dose of 5 mg/kg, as well as in the dose of 50 mg/kg, was accompanied by statistically significant ($p < 0.01$) protective effect. We assume that this effect may by due to nitrate conversion into NO. It is well known that the enzymatic activity of nitrate/nitrite reductases, performing a chain of consecutive conversions, $NO_3^- \longrightarrow NO_2^- \longrightarrow NO$, increases during brain hypoxia or ischemia [1, 9]. Being a strong vasodilator, NO can stimulate blood flow in brain and inhibit platelet aggregation [24]. NO protective effect reveals itself in less pronounced neurological deficit and decreased lethality. Moderate increase in NO concentration leads to proteins shift from soluble state to membrane-associated state, which its turn may make both membranes and proteins more stable. The protective effect of $Mg(NO_3)_2$ in brain ischemia is connected not only with NO production but also with Mg^{2+} cation functions. It is of common knowledge that Mg^{2+} ions are able to enhance the synthesis of prostaglandin I_2, which, in turn, inhibits platelet aggregation. Mg^{2+} cations block calcium channels, thus limiting excessive Ca^{2+} ions flow into the cells, inhibit NMDA receptors, thereby decreasing excitotoxicity, contribute to early recovery of cellular ATP resources, and inhibit proinflammatory cytokine activation, which can reduce damaging effects of brain ischemia and hypoxia [16, 17].

Less pronounced protective action of $Mg(NO_3)_2$ when administered in the dose of 5 mg/kg, compared

to the dose of 50 mg/kg, may be attributed to lower concentrations of both Mg^{2+} cations and NO_3^- anions. In our experiments, when sodium nitrate, $NaNO_3$, was injected in the doses of 5 mg/kg and 50 mg/kg, we also observed the dependence of the rate of nitrate protective action from the administered dose of the compound. $NaNO_3$ administered in the dose of 50 mg/kg significantly reduced neurological deficit and lethality in rats, while $NaNO_3$ administered in a ten fold less dose (5 mg/kg) showed no protective effect [26].

It can be seen in Fig. 1–4, that, during the whole time of the experiment, neurological deficit in the case of $Mg(NO_3)_2$ administration was less pronounced than in the control, but the neurological symptoms become statistically less evident ($p < 0.01$) only 3–4 h after the start of observations. It seems possible that the delay in protective action of $Mg(NO_3)_2$ on the incomplete global brain ischemia may be caused by low nitrate/nitrite reducing activity of Mg^{2+} cation and, consequently, low level of NO production, which, together with Mg^{2+} ions, executed the protector action of $Mg(NO_3)_2$. It takes some time to accumulate the sufficient level of NO for it to exert a moderate protective effect. Potentiating protector cooperation between NO and Mg^{2+}played, possibly, some role too. Other inorganic magnesium compounds, namely $MgSO_4$ and $MgCl_2$, also contained Mg^{2+} cation, nevertheless they had no protective effects in brain ischemia.

Low-level, statistically insignificant protective effect, observed in the case of $MgSO_4$and $MgCl_2$ administration in the doses of 5 mg/kg and 50 mg/kg, seems to be caused by Mg^{2+} cations. The results obtained in the course of the experiment lead us to assume that protector effect of $Mg(NO_3)_2$ does no depend on the time of administration. As can be seen in Figs. 1 and 2, $Mg(NO_3)_2$ injection both 60 min prior to the occlusion of common carotid arteries and 1–2 s after the ischemia demonstrated statistically significant protective effect.

To conclude, the results of our study suggest that pronounced protective effect of $Mg(NO_3)_2$ in the doses of 5 mg/kg and 50 mg/kg on incomplete brain ischemia was caused by the combined action of Mg^{2+} cation and NO_3^- anion. It seem possible that the lack of success while using inorganic magnesium salts, namely $MgSO_4$ and $MgCl_2$, in medicine and experimental work may be accounted for by the action of magnesium compensating ion, that is SO_4^- or Cl^- anion. We assume that the ongoing medical and experimental study of the therapeutic effects of inorganic magnesium compounds will be devoted to understanding the effects of $Mg(NO_3)_2$. Magnesium nitrate meets many of the requirements applicable to the ideal pharmaceutical agent, namely being cheap and accessible, easy in administration, and, apparently, without unfavorable side effects.

REFERENCES

1. Calvert, J.W. and Lefer, D.J., Clinical translation of nitrite therapy for cardiovascular diseases, *Nitric Oxide*, 2010, vol. 22, no. 2, pp. 91–97.
2. Lundberg, J.O. and Weitzberg, E., No-synthase independent no generation in mammals, *Biochem. Biophys. Res. Commun.*, 2010, vol. 396, no. 1, pp. 39–45.
3. L'hirondel, J. and L'hirondel, J.-L., *Nitrate and Man: Toxic, Harmless or Beneficial?* Wallingford: CABI Publ., 2001, p. 184.
4. Pluta, R.M., Oldfield, E.H., Bakhtian, K.D., Fathi, A.R., Smith, R.K., DeVroom, H.L., Nahavandi, M., Woo, S., Figg, W.D., and Lonser, R.R., Safety and feasibility of long-term intravenous sodium nitrite infusion in healthy volunteers, *PLoS One*, 2011, vol. 6, no. 1, p. 14504.
5. Gupta, V.K., Intravenous magnesium for neuroprotection in acute stroke: clinical hope versus basic neuropharmacology, *Stroke*, 2004, vol. 35, pp. 2758–2759.
6. Kaste, M. and Norrving, B., From the world stroke day to the world stroke campaign: one in six: act now!, *Int. J. Stroke*, 2010, vol. 5, pp. 342–373.
7. Hord, N.G., Tang, Y., and Bryan, N.S., Food sources of nitrates and nitrites: the physiologic context for potential health benefits, *Am. J. Clin. Nutr.*, 2009, vol. 90, no. 1, pp. 1–10.
8. Dezfulian, C., Raat, N.J.H., Shiva, S., and Gladwin, M.T., Role of the anion nitrite in ischemia–reperfusion cytoprotection and therapeutics, *Cardiovasc. Res.*, 2007, vol. 75, no. 2, pp. 327–338.
9. Reutov, V.P., Sorokina, E.G., Okhotin, V.E., and Kositsin, N.S., *Tsiklicheskie prevrashcheniya oksida azota v organizme mlekopitayushchikh* (Cyclic Conversion of Nitric Oxide in Mammals), Moscow: Nauka, 1997.
10. Machha, A. and Schechter, A.N., Dietary nitrite and nitrate: a review of potential mechanisms of cardiovascular benefits, *Eur. J. Nutr.*, 2011, vol. 50, no. 5, pp. 293–303.
11. Wink, D.A. and Paolocci, N., Mother was right: eat your vegetables and do not spit! When oral nitrate helps with high blood pressure, *Hypertension,* 2008, vol. 51, pp. 617–619.
12. Bryan, N.S., Cardioprotective actions of nitrite therapy and dietary considerations, *Front. Biosci*, 2009, vol. 14, pp. 4793–4808.
13. Sen, A.P. and Gulati, A., Use of magnesium in traumatic brain injury, *Neurotherapeutics*, 2010, vol. 7, pp. 91–99.
14. Ginsberg, M.D., Neuroprotection for ischemic stroke: past, present and future, *Neuropharmacology*, 2008, vol. 55, no. 3, pp. 363–389.
15. Gums, J.G., Magnesium in cardiovascular and other disorders, *Am. J. Health Sust. Pharm.*, 2004, vol. 61, no. 15, pp. 1569–1576.
16. Muir, K.W., Magnesium in stroke treatment, *Postgrad. Med. J.*, 2002, vol. 78, pp. 641–645.

17. Gromova, O.A., Torshin, I.Yu., Kalachaeva, A.G., and Kuramshin, D.B., Molecular biological basis of neuroprotective effects of magnesium, *Zh. Nevrol. Psikhiatr. im. S.S. Korsakova*, 2011, vol. 111, pp. 12–90.

18. Lin, J.Y., Yang, D.Y., and Cheng, F.C., Experimental cerebral ischemia and magnesium, *Clin. Calcium*, 2004, vol. 14, no. 8, pp. 15–21.

19. Saver, J.L., Targeting the brain: neuroprotection and neurorestoration in ischemic stroke, *Pharmacotherapy*, 2010, vol. 7, no. 2, pp. 62–69.

20. Singh, H., Jalodia, S., Gupta, M.S., Talapatra, P., Gupta, V., and Singh, I., Role of magnesium sulfate in neuroprotection in acute ischemic stroke, *Ann. Indian Acad. Neurol.*, 2012, vol. 15, no. 3, pp. 177–180.

21. Aslanyan, S., Weir, C.J., Muir, K.W., and Lees, K.R., Images study investigators. Magnesium for treatment of acute lacunar stroke syndromes: further analysis of the images trial, *Stroke*, 2007, vol. 38, pp. 1269–1273.

22. Zhu, H.D., Martin, R., Meloni, B., Oltvolgyi, C., Moore, S., Majda, B., and Knuckey, N., Magnesium sulfate fails to reduce infarct volume following transient focal cerebral ischemia in rats, *Neurosci. Res.*, 2004, vol. 49, no. 3, pp. 347–353.

23. Durukan, A., Strbian, D., and Tatlisumak, T., Rodent models of ischemic stroke: a useful tool for stroke drug development, *Curr. Pharm. Des.*, 2008, vol. 14, no. 4, pp. 359–370.

24. Sarkisov, K.Yu., Opits, B., and Oeme, P., Influence of substance P fragment (3–4) on the course of cerebral ischemia in rats with different types of behavior, *Byul. Eksp. Biol. Med.*, 1996, vol. 121, no. 4, pp. 399–403.

25. Iadecola, C., Bright and dark sides of nitric oxide in ischemia brain injury, *Trend. Neurosci.*, 1997, vol. 20, pp. 132–139.

26. Kouzenkov, V.S., Krushinsky, A.L., and Reutov, V.P., Sodium nitrate effect on development of neurological deficiency in rat after semi-global brain ischemia, *Moscow Univ. Biol. Sci. Bull.*, 2011, vol. 66, no. 1, pp. 1–5.

Translated by E. Martynova