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Effect of Magnesium Sulfate on Hypoxic-Ischemic Brain Injury in Newborn Rats with Narrow Neuroprotective Dose-Range

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ABSTRACT

In this study, we examined the neuroprotective effect of magnesium sulfate, which is a potent blocker against NMDA receptor, in postnatal day 7 rats after hypoxia-ischemia. Magnesium sulfate pretreatment at 300~450 mg/kg reduced both the incidence and the size of cerebral infarction ($p < 0.05$). However, either at high (600 mg/kg) or at low (150 mg/kg) dose of magnesium sulfate failed to exhibit significant neuroprotective effect. In addition, the high dose of magnesium sulfate (600 mg/kg) evoked high lethality. These results indicated that neuroprotective effect of magnesium was within the narrow range (300~450 mg/kg) for postnatal rats.

Key words: Magnesium sulfate, cerebral hypoxia-ischemia, cerebral infarction, neuroprotection, newborn rats

INTRODUCTION

Magnesium sulfate has been used in obstetric practice for over 60 years to prevent seizures from preeclampsia-eclampsia and for tocolysis in preterm labor. Recently, population-based reports have focused considerable amounts of interest on the protective effects of magnesium sulfate against cerebral palsy in premature infants whose mothers were treated with magnesium sulfate (Nelson and Grether, 1995). These observations have suggested that magnesium treatment might reduce the incidence of brain injury in perinatal hypoxia-ischemia. However, controversially, other studies failed to demonstrate the neuroprotective effects of magnesium pretreatment with different perinatal animal models (Chi et al., 1990; Hoffman et al., 1994; de Haan et al., 1997). Possible explanations for such controversies might be due to the differences in dosage, timing of application, and animal models. Some retrospective clinical studies also presented controversial issues against neuroprotective effect of magnesium sulfate (Goldenberg and Rouse, 1997; Leviton et al., 1997; O'Shea et al., 1992).

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Because magnesium is known to be a potent antagonist for the NMDA receptor, anti-glutamatergic influence is believed to mediate the neuroprotective effect of magnesium treatment. However, because excitatory amino acids are essential to neuronal growth, synaptogenesis and plasticity in the developing brain, there has been some reservation to use NMDA receptor antagonists clinically (Ikonomidou et al., 1997). Therefore, the present study was undertaken to evaluate the neuroprotective efficacy and safe dosage range for magnesium sulfate, before and after hypoxia- ischemia in postnatal day 7 (P7) rats whose brains are histologically similar to those of human brains at 32～34 weeks of gestation.

MATERIALS AND METHODS

Animals and surgery

Sprague-Dawley rats (P7) were isoflurane-anesthetized, the right carotid arteries of the animals were isolated and ligated, and 1 h later the animals were exposed to 8% oxygen (balanced with nitrogen) for 2.75 h in temperature-controlled (36.5°C) glass chambers. The rats were divided into 2 groups (pretreatment and posttreatment), and each of the groups was divided into 2 sub-groups (magnesium treated and saline control).

To test the efficacy of prophylactic effect, rats were received magnesium sulfate (150, 300, 450, 600 mg/kg/dose) intraperitoneally twice, immediately before and after the induction of hypoxia, which were referred as 'pretreatment group'. To the posttreatment group of rats, magnesium sulfate (450 or 600 mg/kg/dose) were given twice, the first injection immediately after hypoxia-ischemia, and the second injection 2 h after hypoxia-ischemia. Since hypothermia may attenuate cerebral ischemic injury, posthypoxic skin temperature was monitored regularly in a subset of pretreated rats for 3 h after hypoxia, and we found there were no significant differences in weight and nor skin temperatures among the groups. All experimental procedures using animals were in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by the Use of Laboratory Animal Committee of Korea University College of Medicine.

Histological evaluations

To evaluate histopathologic findings, rats were decapitated and brains were rapidly dissected and frozen under dry ice 5 d after the treatment (P12). Frozen brains were coronally sectioned (20μm), post-fixed with 4% paraformaldehyde, and stained with cresyl violet. A computerized image analysis system with NIH Image v1.60 freeware (developed at the U.S. National Institute of Health and available on the Internet at http://rsb.info.nih.gov/nih- image/) was used to measure hemispheric cross sectional areas.

RESULTS

Right carotid artery ligation followed by exposure to 8% oxygen for 2.75 h resulted in an 87.1% incidence of ipsilateral liquefactive cerebral infarction in the saline control group (Table 1). When infarction was detected by gross examination, typical histopathologic findings of cortical and striatal infarctions were well correlated, with pallor, and cortical, striatal, hippocampal, and thalamic atrophy. In the most severely damaged brain, the majority of right hemisphere was liquefied, and few anatomic landmarks were recognizable (Fig. 1A, B). Pretreatment with 150, 300, 450 or 600 mg/kg/dose magnesium sulfate resulted in 83.3% (5/6), 57.9% (11/19), 22.2% (2/9), 66.7% (4/6) incidence of ipsilateral liquefactive cerebral infarction in survivors,

Table 1. Incidence of cerebral infarction by hypoxia-ischemia following magnesium sulfate pretreatment in P7 rats

$MgSO_4$ (mg/kg)	150		300		450		600	
Group (number)	Con (6)	Mg (7)	Con (15)	Mg (19)	Con (13)	Mg (13)	Con (6)	Mg (14)
Cerebral infarction	4	5	12	11	8	2	4	4
No infarction	0	1	2	8	1	7*	1	2
Death	2	1	1	0	4	4	1	8

Con, Control (Saline); Mg, Magnesium sulfate. *$p < 0.05$, compared to the saline control group, Fisher's exact test.

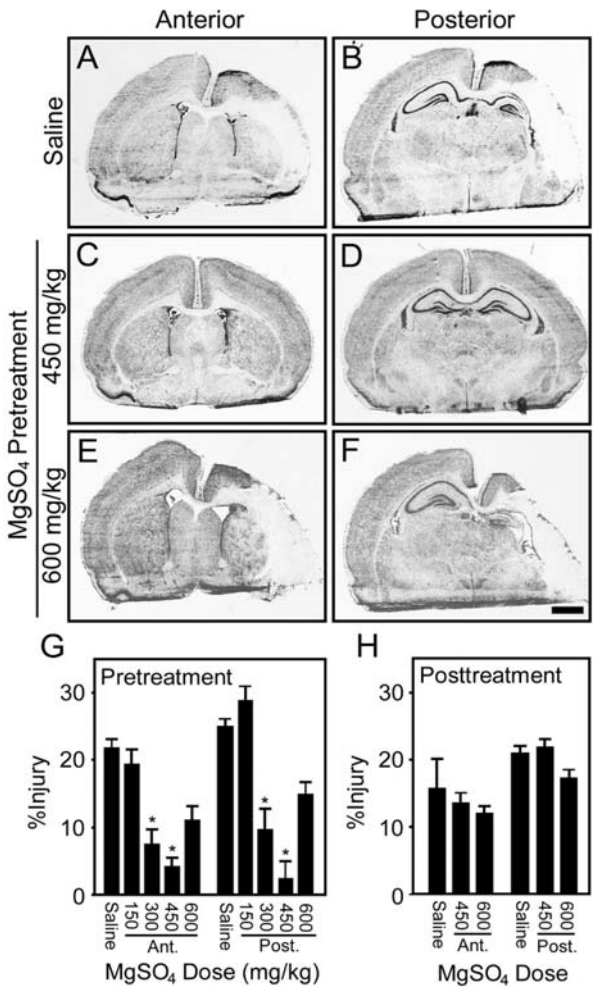

Fig. 1. Dose-dependent neuroprotective effect of magnesium sulfate. Nissl stained brain sections at anterior (A, C, E) and posterior (B, D, F) levels after right cerebral hypoxia- ischemia with saline (A, B), 450 mg/kg (C, D) or 600 mg/kg (E, F) $MgSO_4$ pretreatment. Bar indicates 1 mm. Quantification of neuroprotective effect of $MgSO_4$ was measured in pretreatment (G) and posttreatment (H) groups. The number of animals included in this analysis was described in Tables 1 and 2. Abbreviations: Ant, anterior; Post, posterior (*$P < 0.05$).

respectively (Table 1). Pretreatment group with 450 mg/kg/dose magnesium sulfate resulted in the same incidence of mortality with the concurrent control, whereas the pretreatment with 600 mg/kg/dose resulted in higher mortality (57%, 8/14) than concurrent control (16.6%, 1/6). Quantification of hemispheric areas at two anatomic levels in the magnesium sulfate pretreated groups (150, 300, 450 and 600 mg/kg) demonstrated that the brain damage induced by hypoxia-ischemia was significantly attenuated in rats receiving 300 or 450 mg/kg magnesium sulfate, compared to the control littermates (Fig. 1C, D, G) However, survivors in higher dose (600 mg/kg) of magnesium sulfate treated group failed to exhibit significant neuroprotective effect (Fig. 1E, F, G). Post-hypoxic-ischemic treatment with magnesium sulfate (450 or 600 mg/kg) also failed to attenuate both incidence (Table 2) and the size of cerebral infarction (Fig. 1H) at P12.

Table 2. Incidence of cerebral infarction by hypoxia-ischemia following magnesium sulfate posttreatment in P7 rats

$MgSO_4$ (mg/kg)	450		600	
Group (number)	Con (15)	Mg (18)	Con (16)	Mg (19)
Cerebral infarction	13	13	13	14
No infarction	2	4	2	1
Death	0	1	1	4

Con, Control (Saline solution); Mg, Magnesium sulfate.

DISCUSSION

The release of excitatory amino acid neurotransmitters and recurrent ischemic depolarizations trigger a metabolic cascade, which leads to cell death in a zone surrounding the irreversible infarction core area. The developing brain may be particularly susceptible to the adverse effects of excitatory amino acid receptor overactivation (Barks and Silverstein, 1992), perhaps because NMDA receptor densities are higher than in the mature brain (Johnston et al., 1991). Magnesium, a voltage-dependent non- competitive antagonist of the NMDA receptor, is able to block the lethal calcium influx into the cell when the excitotoxic cascade is overactivated (Nowalk et al., 1984). Furthermore, magnesium acts as a Ca^{2+} antagonist in presynaptic membrane, which can inhibit neurotransmitter release and reduce seizure activity at high concentration (Kass and Lipton, 1982; Rothman, 1983).

We found substantial neuroprotection following pretreatment with magnesium sulfate in the narrow effective dose range. There are several reports demonstrating that a single dose of magnesium sulfate exhibited neuroprotective effect on immature rats (Spandou et al., 1995). Similarly, we also observed that pretreatment with magnesium sulfate on P7 decreased the incidence and severity of brain

damage in the animal model of perinatal cerebral hypoxia-ischemia; modest degree at 300 mg/kg and greater extent at 450 mg/kg dose. However, either higher or lower dose of magnesium sulfate failed to exhibit significant neuroprotective effect. Furthermore, high dose (600 mg/kg/dose) magnesium sulfate also increased the mortality and the dying P7 rats became apneic, with no visible seizures preceding apnea. There was a report demonstrating that 300 mg/kg was the maximum dose at which the general condition of P5 rats could be maintained (Nakajima et al., 1997).

Previous studies suggested that magnesium administration subsequent to hypoxic insults could reduce NMDA-mediated brain injury in perinatal animals (McDonald et al., 1990; Marret et al., 1995), whereas other studies failed to demonstrate substantial improvement with magnesium sulfate following the insult (Penrice et al., 1997; Galvavin and Oorchot, 1998). Here we also observed that post-hypoxic-ischemic treatment with magnesium sulfate failed to reduce neuronal damage. Because extracellular glutamate concentrations were shown to be highest during the period of hypoxia, and returned to the basal level during subsequent normoxia (Silverstein et al., 1991), the neuroprotective efficacy of magnesium treatment might have been operating only during the acute phase of the hypoxia with increased extracellular glutamate.

Our results are consistent with the hypothesis that magnesium pretreatment can attenuate hypoxic-ischemic damage to the immature brain. However, it should be pointed out that the lack of neuroprotective effect by magnesium treatment in certain cases of post-hypoxic-ischemic situations, as well as the relatively narrow safety range of magnesium dosage, suggesting that precautions are required for clinical trials with magnesium for the post-hypoxic-ischemic rescue.

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