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Intrathecal Magnesium Sulfate Administration at the Time of Experimental Ischemia Improves Neurological Functioning by Reducing Acute and Delayed Loss of Motor Neurons in the Spinal Cord

Walter S. Jellish, M.D., Ph.D., Xin Zhang, M.D.,† Kenneth E. Langen, M.D.,‡ Matthew S. Spector, M.D.,§ Michael T. Scalfani, B.S.,‖ Fletcher A. White, Ph.D.#*

Background: **In this study, the authors determined the effect of magnesium sulfate on intrathecal glutamate concentrations, hindlimb motor function, and histopathology after a transient episode of spinal cord ischemia.**

Methods: **Fifty-two New Zealand White rabbits underwent spinal cord ischemia for 30 min. Fifteen minutes before ischemia, animals received intrathecal magnesium sulfate ($MgSO_4$) (3 mg/kg) or placebo (artificial cerebrospinal fluid). Intrathecal microdialysis samples were measured for glutamate using high-performance liquid chromatography. Neurologic function and spinal cord histopathology were assessed throughout the recovery period.**

Results: **Intrathecal glutamate levels in placebo-treated animals were higher after spinal cord ischemia compared with sham- and $MgSO_4$-treated animals. $MgSO_4$-treated animals had increased lower extremity motor function compared with the placebo group (64.7% *vs* 14.3%, $P < 0.01$). Histologic examination of placebo-treated animals revealed significant motor neuron cell loss at thoracolumbar levels by Day 7 ($P < 0.05$), whereas lower lumbar regions displayed significant neuron loss on Day 1. Spinal cords from $MgSO_4$–treated animals exhibited less neuronal loss in lumbar regions. Similar effects were present in the thoracolumbar segments on Day 7. A significant correlation existed between diminished neuronal loss and hind leg movement (Tarlov score) and demonstrates that the neurologic outcome after $MgSO_4$ treatment was related to lower lumbar ventral horn cell survival ($r^2 = 0.812$, $P < 0.001$).**

Conclusions: **These results demonstrate that $MgSO_4$ affords significant spinal cord motor neuron protection by diminishing acute neuronal loss at the foci of the ischemic injury (L3–L6) with delayed neuronal degeneration in adjacent spinal cord regions (T7–L2).**

SPINAL cord ischemia remains a devastating complication after repair of thoracoabdominal aortic aneurysms. Spinal cord motor neurons are especially sensitive to mild or short ischemia.[1,2] This type of injury results in paraplegia.[3] Growing evidence suggests that release of excitatory neurotransmitters, into the extracellular space of the central nervous system by ischemic cells, may contribute to cell death in both cerebral and spinal cord injury.[4–8] There have been extensive studies of various drugs, including postsynaptic N-methyl-D-aspartate (NMDA) receptor antagonists, as possible neuroprotectants after hypoperfusion or ischemia.[9,10]

Magnesium sulfate, a noncompetitive NMDA receptor antagonist, has demonstrated a variety of neuroprotective actions in the central nervous system after induction of experimental ischemia[11–14] and in clinical studies.[15] The majority of these studies have focused on how magnesium prevented or attenuated necrotic phenomenon in the gray matter of spinal cord, and preserved motor function partially or completely. This antagonistic action is speculated to occur from a direct postsynaptic effect. However, the effects of magnesium on the extracellular concentration of glutamate during a spinal cord ischemic event have not been demonstrated *in vivo*.

In the present study, we sought to determine the degree to which magnesium sulfate affects the concentration of glutamate in cerebrospinal fluid and enhances motor neuron survival in both the foci and the penumbra of spinal cord experimental ischemia. By investigating the relationship between motor neuron survival after a combination of ischemia and magnesium sulfate ($MgSO_4$) at different levels of the spinal cord, we provide evidence that the beneficial effects of a one-time administration of $MgSO_4$ are not limited to only the ischemic lesion foci but can also affect neuronal degeneration several days later in adjacent penumbral nervous tissue.

Materials and Methods

Animal Subjects

After approval by our institutional animal care and use committee (Loyola University Medical Center, Maywood, IL) with adherence to American Physiology Society/National Institute Health guidelines, male New Zealand rabbits (n = 52) weighing 2–3 kg at the beginning of

* Professor, † Research Assistant Professor, ‡ Resident, § Medical Student, Department of Anesthesiology, Loyola University Medical Center, Maywood, Illinois. ‖ Research Assistant, Department of Cell Biology, Neurobiology and Anatomy, Stritch School of Medicine, Loyola University of Chicago, Maywood, Illinois. # Assistant Professor, Department of Anesthesiology, Loyola University Medical Center and Department of Cell Biology, Neurobiology and Anatomy, Stritch School of Medicine, Loyola University of Chicago.

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Address correspondence to Dr. Jellish: Loyola University Medical Center, 2160 South First Avenue, Building 103 – Room 3114, Maywood, Illinois 60153. wjellis@lumc.edu.

experiments were used. They were housed under controlled laboratory conditions consisting of a 12-h light/dark cycle with lights turned on at 6:00 AM, a temperature of 22 ± 1°C, and 55 ± 5% humidity. Food pellets and water were supplied *ad libitum*. Under anesthesia with isoflurane, a catheter was inserted into the auricular vein and fixed on the ear for drug administration. Saline ($10\ ml \cdot kg^{-1} \cdot h^{-1}$) was administrated intravenously to prevent dehydration. A butterfly catheter (0.5 × 19 mm × 304.8 mm tubing infusion set) filled with heparinized saline was inserted into the auricular artery and fixed on the ear for constant monitoring of blood pressure and heart rate during surgery. The rectal temperature of animals was maintained between 37 and 37.5°C with a heating pad before and after aortic occlusion.

Experimental Design

A total of 52 rabbits were randomly assigned to one of seven treatment groups with 6–10 animals per group. One group of animals served as sham controls by undergoing the surgical procedure without aortic occlusion followed by 24 h of survival. The other six groups underwent a 30-min aortic artery occlusion followed by a survival time of 1, 2, or 7 days. Three of these groups (1, 2, and 7 d postoperative survival) received placebo treatment, which consisted of artificial cerebrospinal fluid intrathecal administration 15 min before the occlusion. The remaining three groups received $MgSO_4$ intrathecal administration 15 min before the occlusion and were allowed to survive for either 1, 2, or 7 days.

Experimentally Induced Spinal Cord Ischemia

Anesthesia. Rabbits were anesthetized *via* an intravenous sedative agent, propofol (10 mg/kg). A mask was placed over the rabbit's muzzle for further induction of anesthesia using isoflurane 4% in oxygen; tracheal intubation was then performed. The lungs were mechanically ventilated with isoflurane 1–3% in oxygen throughout the surgery and experimental procedures to maintain anesthesia.

Aortic Occlusion. Spinal cord ischemic injury was produced according to a previously described method.[4,13,16] Briefly, the abdominal cavity was entered through a midline incision, and the aorta was exposed at the level of the left renal artery. The artery was then isolated 1 cm below the renal bifurcation. A polyethylene suture (PE10 tubing) was passed underneath the aorta, and a button combined with larger plastic tubing (PE190, 10 cm in length) was used to protect the aortic artery wall during ligation. The abdominal incision was then sutured. Arterial occlusion was achieved by pulling the suture outside the abdomen to obstruct blood flow for 30 min. A Doppler probe flow device (Ultrasonic Flow Detectors Models 811-B/811-BTS; Medical Electronics, Inc., Aloha, OR) pointing down against the skin surface of the groin area was used for repeated measuring of the femoral artery pulse to ascertain confirmation of occlusion and reperfusion. Heparin 150 U/kg was administered intravenously 5 min before aortic occlusion to prevent clot formation. The sham procedure involved conducting a similar surgical procedure without aortic occlusion. Surgery was performed with aseptic technique.

Experiment 1: Effect of $MgSO_4$ on Cerebral Spinal Fluid Levels of Glutamate. This experiment examined the effects of $MgSO_4$ on the level of glutamate present in the cerebral spinal fluid before (60 min), during (30 min), and after the period of aortic occlusion (150 min). Rabbits were randomly assigned to undergo either $MgSO_4$ treatment (3 mg/0.05 ml/kg) or placebo (artificial cerebral spinal fluid) (n = 12 rabbits placebo plus occlusion; n = 12 $MgSO_4$ plus occlusion; n = 4 sham-treated controls).

Spinal Cord Microdialysis and Microinjection

A microdialysis probe (outside diameter, 200 μm; length, 70 mm) along with microinjection tubing (outside diameter, 152 μm; length, 60 mm) manufactured in our laboratory was inserted through a 22-gauge guide needle placed between lumbar vertebral levels L4 and L5 into the subarachnoid space. The recovery efficiency of the microdialysis probe was approximately 15%, as previously determined *in vitro*. The permeable section of the probe was positioned cephalad between the T12 and L4 levels. During the experiment, artificial cerebrospinal fluid (125 mM NaCl, 0.86 mM $CaCl_2$, 3 mM KCl, 0.89 mM $MgCl_2$, 25 nM $NaHCO_3$, 0.5 nM NaH_2PO_4, and 0.25 nM Na_2HPO_4), which served as a perfusion medium, was infused *via* a syringe pump at a rate of 3 μl/min to collect perfusate before and after drug treatment and at onset and offset of ligation procedure. Each sample was collected for 30 min, and two samples were collected at baseline before aortic occlusion. The duration of total collection was 4 h. $MgSO_4$ (3 mg/0.05 ml/kg) or placebo (artificial cerebrospinal fluid), which was determined based on a previously reported study on dogs,[13] was injected intrathecally with a syringe pump set at 0.01 ml/min. The total duration of $MgSO_4$ infusion was based on the body weight (5 min/kg) and was performed approximately 15 min before the onset of ischemia.

Recovery

Each experiment lasted between 6 and 7 h. At the end of the experiment, the arterial and venous lines were removed. Anesthetic administration was discontinued, and the lungs were ventilated with 100% oxygen. Extubation of the trachea took place when vigorous spontaneous ventilation and movement occurred. After extubation and cessation of the experiment, the animal was randomly assigned to recover for 1, 2, or 7 days.

Cephazolin 10 mg/kg intramuscularly was administered once per day for 2 d to prevent infection, and lactated Ringer's solution 40 ml was administered subcutaneously to prevent dehydration. Buprenorphine 0.03 mg/kg was given subcutaneous to control pain twice per day. If animals showed signs of pain, such as grooming or guarding, 2 d after surgery, then additional pain medication was administered. Bladder contents were expressed manually twice per day.

Chemical Analysis

Measurement of glutamate from the spinal cord dialysate was performed using high-performance liquid chromatography (Waters Corp., Milford, MA).[17] The system consists of a solvent delivery system (Waters 515 HPLC pump, Waters Corp.), an octadecyl disufonate C_{18} 3 Fm 100 × 3.2-mm phase II column, and a fluorescent detector (Waters 474, Water Corp.) with excitement set at 330 λ and emission set at 425 λ. The mobile phase consisted of 0.1 M sodium acetate in 10% acetronitrile, pH 6.8 as buffer I and 70% acetronile as buffer II. Precolumn derivatization with O-phthalaldehyde reagent solution was performed manually. After derivatization, each standard solution and prepared sample was injected into the system. Glutamate and aspartate samples in a standard solution and biologic samples were separated as they migrated along the phase II column (retention times, Asp = 2.69 min; glutamate 4.65 min.). Biologic samples were quantified against a standard curve of known solutions run with each sample set. The area of glutamate peak was converted to an actual concentration (picomole/microliter) using linear regression curves obtained from the series of standard samples. The baseline concentration was calculated by averaging the actual concentration of each component in the initial two 30-min samples.

Experiment 2: Effect of $MgSO_4$ on Neurologic Outcome as Determined by Behavior and Neuronal Survival. This experiment examined the effects of $MgSO_4$ on hind limb motor function and neuron survival after the period of aortic occlusion (see Aortic Occlusion section above). Rabbits were randomly assigned to undergo either $MgSO_4$ treatment (3 mg/0.05 ml/kg) or placebo (artificial cerebral spinal fluid) (n = 13 rabbits placebo plus occlusion; n = 17 $MgSO_4$ plus occlusion; n = 7 sham-treated controls).

Neurologic Outcome

Animal hind limb motor function was observed and scored daily for 1, 2, or 7 d after the ischemic event. A Tarlov five-point grading scale was used: 4 = normal; 3 = ability to draw legs under body and hop, but not normal; 2 = some lower extremity function with good antigravity strength but inability to draw legs under body; 1 = poor lower extremity motor function, weak antigravity movement only; and 0 = paraplegic with no lower extremity motor function muscle tone or contraction. The experimenter was blinded as to the condition of the animal.

Histology and Cell Counting Analysis

Rabbits were euthanized by an overdose of pentobarbital 100 mg/kg intravenously 1, 2, or 7 d after the ischemic event,. The spinal cord tissue was fixed by transcardial perfusion of 4% paraformaldehyde and processed for paraffin embedding. Four sections from each spinal cord level (T7–L5) were stained with Nissl stain and photographed on a light microscope (BH2-RFCA; Olympus, Center Valley, PA). Neurons were distinguished within the spinal cord ventral horn based on cell morphology, a clear nucleus, and prominent nucleolus. The ventral horn cells with a clear nucleus were counted and traced. There was a 400-μM distance between each stained tissue section. Based on spinal cord levels of hind limb muscular innervation, spinal cord levels L3–L6 were pooled together, as were spinal cord levels of non–hindlimb-associated spinal cord segments, T7-L2. Results are presented as mean ± SEM of the total cells in the two prespecified regions of spinal cord segments. The experimenter was blinded as to the treatment condition of the animal.

Statistical Analysis

SAS software (SAS Institute Inc., Cary, NC) was used for the following statistical analyses. A repeated-measures analysis of variance was used to determine whether glutamate levels present in spinal cord dialysate differed among treatment groups and differed within subjects over time. A Fisher exact test was performed to analyze differences among the neurologic behavioral outcome groups. For the cell counting analysis, a SAS code was created and applied to the raw data to sort and group the data based on the cell size range in each spinal cord segment. The frequency averages and standard deviations were computed for each subgroup. After grouping results based on the variable treatments, a nonparametric Wilcoxon rank sums test was performed to compare the significance within the region among the treatment groups using χ^2 analysis. The paired *t* test compared the mean of the differences in the observations between two regions. Linear regression analysis was using to compare hindlimb motor function (Tarlov scores) with cell survival (cell count of viable cells). Model assumptions (normality and equality of variances) were assessed using graphical (*i.e.*, q-q plots) and analytical methods (*i.e.*, kurtosis/skewness-based tests[18] for normality, and Bartlett's test[19] to verify equality of variances among groups).

Table 1. Hemodynamic Changes during Spinal Cord Ischemia

	Placebo (n = 21)			$MgSO_4$ (n = 24)			Sham (n = 7)		
Aortic Occlusion	Before	During	After	Before	During	After	Before	During	After
MBP, mm Hg	41 ± 1.60	44 ± 1.78	43 ± 1.53	45 ± 2.33	38 ± 2.43	40 ± 2.15	40 ± 3.97	38 ± 4.99	39 ± 2.79
HR, beats/min	228 ± 5.41	228 ± 4.90	226 ± 5.85	236 ± 4.98	229 ± 4.81	219 ± 4.07	231 ± 2.35	231 ± 5.16	236 ± 3.63
Body temperature, °C	37.2 ± 0.13	36.8 ± 0.15*	37 ± 0.19	37.3 ± 0.14	36.8 ± 0.13†	37 ± 0.14	37.1 ± 0.35	37.2 ± 0.35	37.7 ± 0.43
Spo_2, %	98 ± 0.21	98 ± 0.20	98 ± 0.19	98 ± 0.17	98 ± 0.23	98 ± 0.14	98 ± 0.37	98 ± 0.20	98 ± 0.25
Glucose, mg/dL	182 ± 16.07	152 ± 28.11		193 ± 17.00	154 ± 8.99		159 ± 23.10	151 ± 28.17	

Data are presented as mean ± SEM.

* $P = 0.045$ and † $P = 0.013$ compared with the before occlusion value.

HR = heart rate; MBP = mean blood pressure; Spo_2 = percentage of oxygen saturation.

Results

Experiment 1

Physiologic values are presented in table 1. There were no physiologically relevant differences among the groups for all monitored variables with the exception of rectal temperatures. Placebo- and $MgSO_4$-treated rabbits showed similar values in mean blood pressure, heart rate, and blood glucose levels. The rectal temperatures, however, were significantly reduced 0.4-0.5°C during aortic artery occlusion in placebo- and $MgSO_4$-treated groups compared with the sham group, but they returned to the preocclusion baseline level after reperfusion (table 1).

Before the ischemic challenge, baseline levels (mean ± SEM) of glutamate were similar in all three groups (2.13 ± 0.18 μM in the placebo group, 2.65 ± 0.41 μM in the $MgSO_4$ group, and 3.31 ± 1.23 μM in the sham group). The baseline values were considered as 100%, and each animal served as its own control. Glutamate levels in cerebrospinal fluid increased by 212% immediately after ischemia plus placebo and remained elevated for at least 2 h ($P = 0.0001$) after the offset of ischemia (n = 12) (fig. 1). In contrast, pretreatment with intrathecal $MgSO_4$ before ischemia produced glutamate levels similar to those recorded for sham-injured animals (n = 12 and n = 4, respectively) (fig. 1).

Experiment 2

Animals subjected to all treatment conditions were neurologically evaluated on a daily basis using a Tarlov five-point scoring system. Of the placebo-treated animals, 86% exhibited complete paraplegia with rigidly extended hind limbs and no movement (Tarlov 0) (fig. 2). The remaining animals had slight lower extremity function (Tarlov 1). $MgSO_4$-treated animals had better neurologic outcomes (Tarlov 0–3). Twelve percent demonstrated good antigravity strength (Tarlov 3), 18% had some antigravity strength (Tarlov 2), 35% had weak antigravity strength (Tarlov 1), and 35% demonstrated Tarlov 0 scores, whereas 100% of the sham animals had completely normal examinations (fig. 2). The data from Days 2 and 7 are presented together in figure 2. The animal's motor function was not different between Day 2 and Day 7. The worst neurologic outcomes had already been obtained (placebo-treated) on Day 2 even for the animals that survived 7 days. Overall, there was a significant difference between sham and either placebo- or MgSO4- treated group. There is also a significant difference when the $MgSO_4$-treated group was compared with the placebo group.

Cells with motor neuron-like morphology were assessed from segments T7 to L6 (fig. 3). Neuronal loss was greatest in the lower lumbar spinal segments L3–L6 on Day 1 in the ischemic group receiving the placebo treatment (fig. 3b). In contrast, animals receiving $MgSO_4$ in combination with ischemic treatment exhibited significantly lower loss of motor neurons on Day 1 compared with the placebo-treated group (fig. 3b).

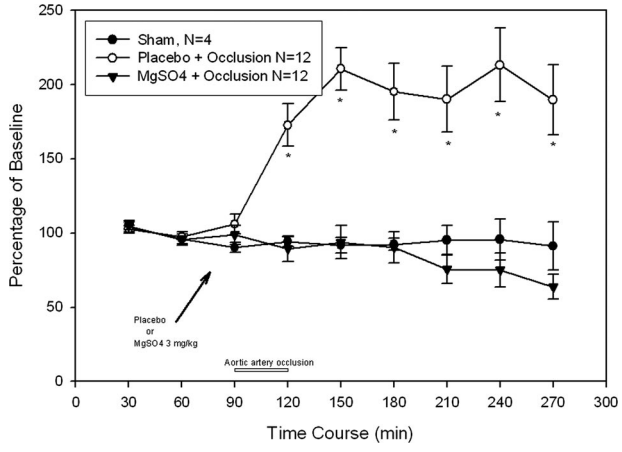

Fig. 1. Effects of intrathecal magnesium sulfate on ischemia-induced release of glutamate in the intrathecal dialysate in rabbits. Data are expressed as mean ± SEM percentage of the mean baseline levels in each group. *$P < 0.05$ when the values in placebo group were compared with the corresponding values in $MgSO_4$ or sham group across the time points. There is also a significant difference ($P < 0.0001$) for the overall glutamate levels after spinal cord ischemia when the placebo group was compared with the $MgSO_4$ or sham group. Sham = animals undergoing surgical procedure without aortic occlusion. Placebo + occlusions = artificial cerebrospinal fluid + occlusion in animals from 1, 2, and 7 d survival. $MgSO_4$ + occlusion = $MgSO_4$ + occlusion in animals for 1, 2, and 7 days survival.

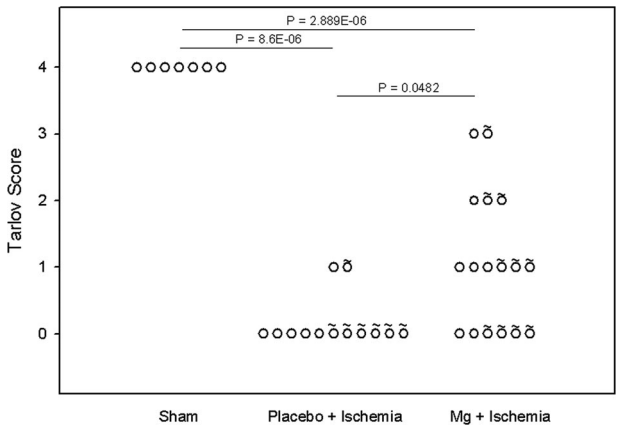

Fig. 2. Tarlov scores were used to evaluate the motor function in each individual rabbit 2 and 7 d after 30-min spinal cord ischemia. The comparisons were made, and *P* values among groups are presented. O = animals that survived 2 days; Õ = animals that survived 7 days. Tarlov score: 0 = paraplegic with no lower extremity motor function, muscle tone, or contraction; 1 = poor lower extremity motor function, weak antigravity movement only; 2 = some lower extremity function with good antigravity strength but inability to draw legs under body; 3 = ability to draw legs under body and hop, but not normal; 4 = normal. We did not assess animals that survived 1 day. Day 2 assessments were unchanged in animals that survived 7 days.

There was a parallel, albeit delayed, loss of neurons in the spinal cord adjacent to L3–L6. Spinal cord levels T7-L2 displayed a loss of neurons by Day 7 (fig. 3a). The differences between the two regions (T7-L2 and L3-L6) were statistically significant in the placebo group (P = 0.0086, 0.0005, and 0.0001, respectively for the three time points). The cell loss present on Day 7 did not approach the degree of damage present in L3–L6. However, the numbers of remaining neurons were significantly lower when compared with sham-treated tissue and Postinjury Days 1 and 2 (fig. 3a). Not unlike the neuroprotective effects in spinal segments L3–L6 in placebo-treated ischemic animals, the addition of $MgSO_4$ just before the ischemic event largely eliminated the loss of neurons in T7-L2 (fig. 4).

Linear regression analysis comparing hind limb motor function (Tarlov scores) including the data from Days 2 and 7 with cell survival (cell count of viable cells) demonstrated a significant coefficient ($r^2 = 0.81$, $P < 0.001$), indicating a considerable relationship between motor function and integrity of motor neurons in the spinal cord (fig. 5).

Discussion

Transient ischemia in the spinal cord arising as a consequence of thoracic surgery, trauma, or ischemia that is experimentally induced elicits increased release of extracellular glutamate. Ischemia-enhanced concentrations of this neurotransmitter may lead to excitotoxicity and neuronal cell death *via* calcium-mediated NMDA receptor-dependent intracellular processes.[10] Spinal cord neu-

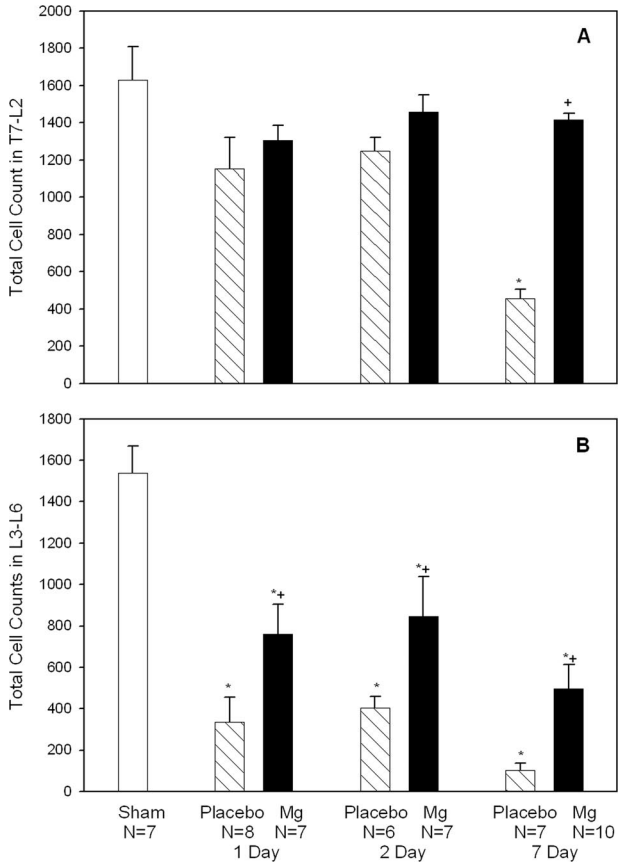

Fig. 3. Effects of intrathecal magnesium sulfate (3 mg/kg) on total cell (>200 μm^2) counts in the ventral horn after 30-min spinal core ischemia. Results are presented as mean ± SEM of the total cells in two regions of spinal cord segments. (*A*) Cell counts in the segments of T7-L2. (*B*) Cell counts in the segments of L3-L6. **P* < 0.05 when the values were compared with the sham control values. +*P* < 0.05 when the values were compared with the corresponding placebo groups. Sham = *open bars*, placebo = *notched bars*. $MgSO_4$ treated = *solid bars.*

rons, especially motor neurons in the ventral horn, are extremely sensitive to excitotoxicity and constitute one of the main neural elements damaged after spinal cord ischemia.[1,3,20,21] In this article, we provide direct evidence that the addition of $MgSO_4$ just before experimentally induced ischemia effectively eliminates injury-induced glutamate increases in cerebrospinal fluid. $MgSO_4$-treated animals subjected to spinal cord ischemia also exhibit significantly increased numbers of motor neurons in both the lumbar and lower thoracic levels of the spinal cord compared with the ischemia/placebo treatment group. In addition, there was a strong linear correlation between decreased neuronal cell death and hindlimb mobility when ischemic animals were pretreated with intrathecal $MgSO_4$.

Our data corroborate results in a variety of animal models[4,5,7,8] and in humans.[6] However, it seems that there are two mechanisms of cell death operating within the ischemic spinal cord, as most of the hindlimb motor neurons present in the lumbar spinal cord are lost with the first 24 h, whereas there is no histologic evidence of

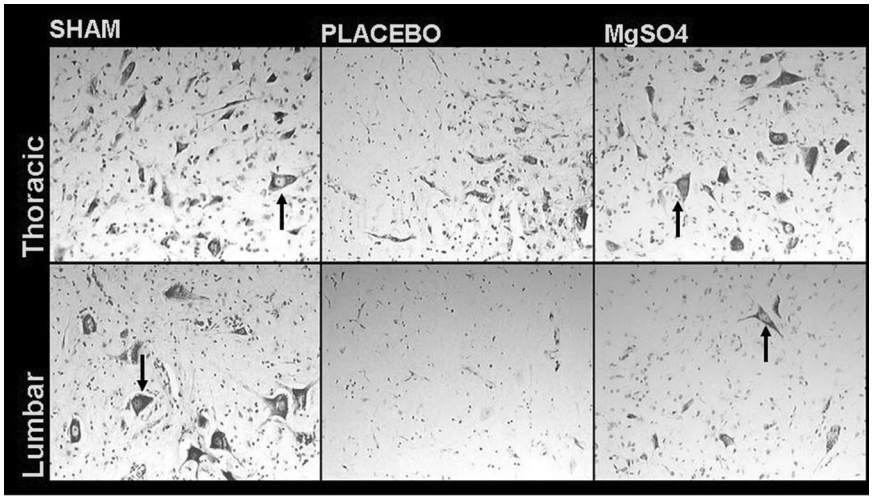

Fig. 4. Representative photomicrograph of cells from a visual field of ventral horn with 10× magnification in the T8 and L4 segments. *Black arrows* indicate the part of Nissl-positive large cells. The size of large cells are 600-2,500 μm^2.

degeneration in the lower thoracic/upper lumbar region of the spinal cord until after 48 h. These differences in regional cell death may not differ from a mixed form of concurrent apoptosis and necrosis in the ischemic cerebrum termed hybrid death.[22–23] Moreover, it would seem that regardless of the mechanism of cell death, a one-time administration of $MgSO_4$ provides significant benefits for both regions of the injured spinal cord.

As there is benefit from $MgSO_4$ administration in both the reduction of cerebrospinal fluid glutamate concentrations and reduced neuronal loss after ischemia, cell death mechanisms are expected to exhibit some dependence on glutamate-specific receptors, such as the calcium (Ca^{2+})-permeable NMDA-type receptors.[9,10] In this regard, the role of $MgSO_4$ is likely as a noncompetitive antagonist of the NMDA receptors present on both pre-

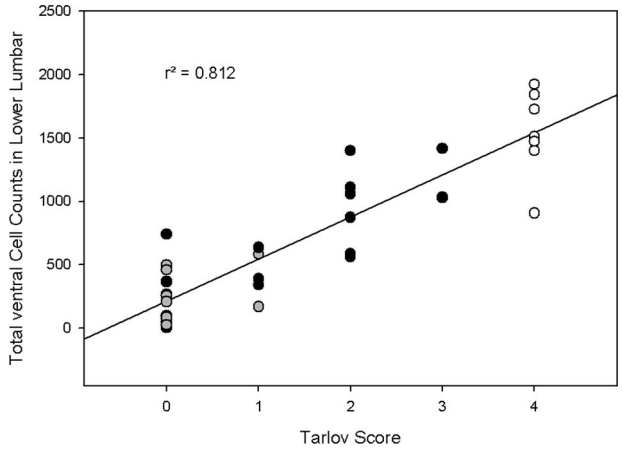

Fig. 5. The relationship between the total number of cells that survived the ischemic event in the ventral horn and motor function Days 2 and 7 after 30-min spinal cord ischemia. Data are expressed as the individual value for each animal. *Blank circles* represent the sham animals, *gray circles* represent placebo plus ischemia animals, and *black circles* represent magnesium plus ischemia animals. The correlation coefficient of this lineal regression was indicated. Data for animals that survived 1 day were not assessed. Day 2 assessments were unchanged in animals that survived 7 days.

synaptic and postsynaptic neurons.[24] Regardless of site of receptor action, it seems that injury-induced increases in glutamate accumulation are likely to overstimulate neurons, leading to a vicious cycle in which massive increases in extracellular glutamate eventually lead to glutamate receptor overactivation (excitotoxicity) and rapid postsynaptic Ca^{2+} accumulation.[25] Evidence of such a condition has been demonstrated using the NMDA antagonist, D-APV, which effectively reduces neuronal calcium accumulation in a manner proportional to its protective effect.[26]

Neuronal cell death after ischemia is not limited to glutamate-mediated influx of calcium *via* ionotrophic NMDA receptors, as there is growing evidence that expression of novel α-amino-3-hydroxymethylisoxazole-4-propionate (AMPA) receptors may also undermine neuronal survival after nervous system ischemic events. Under normal circumstances, it is thought that AMPA receptors mediate the majority of fast excitatory transmission in the mammalian central nervous system *via* Ca^{2+}-impermeable tetrameric assemblies of subunits GluR1-4[27,28] as relatively few neuron populations exhibit Ca^{2+}-permeable AMPA receptors.[29] After global ischemia, numerous groups have demonstrated that pyramidal cells in the hippocampus upregulate expression of AMPA receptors that lack the GluR2 subunit. This particular AMPA receptor isoform is permeable to both Ca^{2+} and Zn^{2+},[30–32] a feature likely to contribute to neuronal cell death.[33]

Recent studies on the susceptibility of neurons to either ischemia or disease pathologies reveal that spinal motor neurons can also exhibit these AMPA receptors lacking GluR2.[34] Moreover, the presence of tumor necrosis factor-α, a pro-inflammatory cytokine typically present during the initial stages of ischemic-induced neuroinflammation, facilitates additional membrane insertion of Ca^{2+}-permeable AMPA channels into motor neurons[35–37] and may profoundly contribute to changes in Ca^{2+}-permeable AMPA receptors after ischemia.[38] Taken

together, glutamate receptor-mediated changes in neuronal levels of Ca^{2+} likely lead to mitochondrial disruption, generation of reactive oxygen species, release of apoptotic mediators such as cytochrome C, and neuronal cell death.[39] Despite a strong potential for attenuation of Ca^{2+} translocation after a combination of ischemia and magnesium, biologically relevant *in vivo* testing has yet to be conducted.

Several mechanisms independent of neuronal glutamate receptors may also be modified in the extracellular space after ischemia, which may contribute to superfluous amounts of glutamate. Normally glutamate released into the extracellular space is quickly removed and transported into the astroglial syncytium by sodium-dependent astrocytic Glu transporters (GLT-1).[40–42] GLT-1 transports one glutamate anion coupled to the co-transport of three Na^+ and one H^+, as well as to the countertransport of one K^+.[43] GLT-1 uses steep ionic gradients across the membrane to accumulate a high intracellular concentration of glutamate in astrocytes. The ionic gradients are mainly maintained by Na^+/K^+-ATPase, which excludes Na^+ in exchange for extracellular K^+, in turn energizing other secondary ion transporters (*e.g.*, Na^+-Ca^{2+} exchanger). After ischemia, changing ionic gradients may negate the driving force for glutamate uptake, resulting in a reversed operation of astrocytic GLTs and release of neuropathological levels of glutamate, potentially contributing to the death of neurons.[44–46]

Not unlike neurons, astrocytes are also known to exhibit both metabotropic and ionotropic glutamate receptors, which may facilitate astrocytic detection of changes in extracellular glutamate levels.[47] However, the effects of magnesium on GLT-1 reversal are not apparent given that the NMDA receptors present on astrocytes seem to be insensitive to the presence of up to 10 mM magnesium.[47]

The benefits of magnesium treatment during ischemia may not be limited to neuron and glial support cells as magnesium produces relaxation of vascular smooth muscle,[48] effectively enhancing cerebral blood flow.[49] The increase in blood flow secondary to vasodilation could improve perfusion to ischemic neurons and effectively reduce glutamate release. This magnesium-dependent mechanism may involve antagonism of the vasoconstrictive mediator, endothelin-1.[50] Magnesium has also been shown to attenuate vasoconstriction induced by norephinphrine, angiotensin II, and serotonin[51,52] and to prevent thrombosis by inhibiting platelet reactivity.[53] Additional evidence that local vasodilation and improvement in blood flow may be a major mechanism in reducing glutamate release is demonstrated by studies that show magnesium has a minimal effect on improving neurologic function if administered peripherally.[54] Magnesium may not penetrate the blood-brain barrier sufficiently to increase cerebrospinal fluid concentrations to a level that would produce local vasodilation. In the present study, magnesium was administered intrathecally and probably produced local concentrations much higher than if given by parenteral infusion. These effects seem beneficial for cell survival after ischemic insult and have an undetermined impact on our study.

Only one animal study[55] has questioned the safety of intrathecal magnesium administration by observing neurotoxicity in a dose-dependent manner including histopathologic changes and motor dysfunction (Tarlov score 1-0) 7 days post-administration in rabbits. This study further demonstrated that intrathecal magnesium (0.3 or 1 mg/kg) did not improve hindlimb motor function and histopathologic outcomes induced by aortic occlusion and that higher concentrations of magnesium (2–3 mg/kg) administered intrathecally caused direct damage on spinal cord neurons. This result is contrary to a majority of reports from magnesium studies in which neuroprotective effects of magnesium have been confirmed in different animal models by different investigators.[11,12] More recent clinical evidence suggests that the use of intravenous magnesium (5 days treatment) did not differ from placebo treatment in individuals with a combination of moderate to severe traumatic brain injury (within 8 h) and low serum magnesium.[56] This outcome is suggestive that the neuroprotective effect of magnesium may be dependent on the route of administration or be limited only to individuals with certain clinical criteria (thoracoabdominal aneurysm repair) or neurologic injury.

Recent studies demonstrated that glutamate-induced calcium entry and energy failure has played a deleterious role for the delayed secondary spinal cord injury. It was reported that the initial excitotoxic events associated with necrosis also activate microglia and produce proinflammatory cytokines, which may be crucial for inducing necrotic and apoptotic cell death. New approaches are focusing on using treatments that target the nonneuronal cellular components of the central nervous system inflammatory response and long-term secondary events after initial injury. It is likely that the key to postischemic treatment will include the control of factors that modulate the inflammatory response.[57–59] However, the effects of $MgSO_4$ and other potential neuroprotective agents on the ischemia-induced inflammatory response are unknown.

Taken together, the present study has demonstrated that a single intrathecal administration of $MgSO_4$ attenuates immediate release of glutamate and reduces neurologic injury after transient ischemia both in the foci and associated pneumbra of the spinal cord. Further investigations will focus on magnesium activation of glutamate transport proteins and inhibition of the inflammatory process.

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