Prevention of ischemic spinal cord injury: Comparative effects of magnesium sulfate and riluzole

Loïc Lang-Lazdunski, MD, Catherine Heurteaux, PhD, Hervé Dupont, MD, Catherine Widmann, BA, and Michel Lazdunski, PhD, DSc, Paris, France

Purpose: Excitotoxic mechanisms have been implicated in the pathophysiology of spinal cord ischemic injury induced by aortic cross-clamping. We investigated the effects of the anti-excitotoxic drugs magnesium sulfate (MgSO₄) and riluzole in a rabbit model of spinal cord ischemia.

Method: The infrarenal aorta of New Zealand albino white rabbits (n = 68) was occluded for 40 minutes. Experimental groups included: a control group, which received only vehicle (n = 17); group A (n = 17), which received riluzole (8 mg/kg) before clamping; group B (n = 17), which received MgSO₄ (100 mg/kg) before clamping; and group C (n = 17), which received riluzole (8 mg/kg) and MgSO₄ (100 mg/kg) before clamping. Five additional rabbits had the same operation, but did not undergo aortic clamping (sham operation). The neurological status of the rabbits was assessed at 24 hours, 48 hours, and then daily for as long as 120 hours by using a modified Tarlov scale. The rabbits were killed at 24 hours (n = 3 per group), 48 hours (n = 4 per group), and 120 hours (n = 10 per group) postoperatively. Spinal cords were harvested for histopathologic and immunohistochemistry examinations for microtubule-associated protein-2 (MAP-2), a cytoskeletal protein specific from neurons.

Results: No major adverse effect was observed with either riluzole or MgSO₄. All control rabbits became severely paraplegic. All riluzole-treated and MgSO₄-treated animals had a better neurological status than control animals. Typical morphological changes characteristic of neuronal necrosis in the gray matter of control animals was demonstrated by means of the histopathological examination, whereas riluzole or magnesium prevented or attenuated necrotic phenomena. Moreover, MAP-2 immunoreactivity was completely lost in control rabbits, whereas it was preserved, either completely or partially, in rabbits treated with riluzole or magnesium. Riluzole was more effective than MgSO₄ in preventing paraplegia caused by motor neuron injury (P < .01). Riluzole and MgSO₄ had no additive neuroprotective effect.

Conclusion: These results demonstrate that riluzole and, to a lesser extent, MgSO₄ may afford significant spinal cord protection in a setting of severe ischemia and may, therefore, be considered for clinical use during “high-risk” operations on the thoracic and thoracoabdominal aorta.

Spinal cord ischemia remains a devastating complication of thoracoabdominal aortic surgery, with paraparesis or paraplegia occurring in as many as 21% of procedures. The pathophysiologic mechanisms that underlie hypoxic/ischemic injury to the spinal cord have not been totally elucidated, but recent studies suggested that the release of excitatory amino acids into the extracellular space of the central nervous system by ischemic cells may play a key role in the neuronal cell death occurring in the ischemic spinal cord. Glutamate and aspartate may destroy neuronal cells through their actions on N-methyl-D-aspartate (NMDA) and non-NMDA receptors by inducing

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Competition of interest: nil.

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massive sodium and calcium ion influxes into the cell, resulting in neuronal death.4

NMDA and non-NMDA receptor antagonists have demonstrated neuroprotective properties both in vitro and in vivo,3-6 but their major adverse effects limit their clinical use.7,8

Riluzole (2-amino-6-trifluoromethoxybenzothiazole) is a neuroprotective drug that blocks glutamatergic neurotransmission in the central nervous system and has demonstrated anti-ischemic properties both in vitro and in vivo.9 A recent experimental study also demonstrated that riluzole may prevent spinal cord ischemic injury induced by aortic cross-clamping.10 Riluzole is currently in clinical use

Fig 1. Histopathological examination (original magnification, 400×). Representative photomicrographs of lumbar spinal cord sections stained with Klüver-Barrera stain from sham-operated (A), control (B), riluzole-treated (C), magnesium-treated (D), and riluzole plus magnesium-treated (E) rabbits that underwent 40 minutes of spinal cord ischemia and 120 hours of reperfusion. The chosen sections for illustration of riluzole-treated and riluzole plus magnesium-treated groups (groups A and C) correspond to rabbits with a Tarlov score of 5. The chosen section for illustration of the magnesium-treated group (group B) corresponds to a rabbit with a Tarlov score of 2. A, Normal appearance of motor neurons in the ventral horn of a sham-operated rabbit. B, Necrotic motor neurons in the ventral horn of a control rabbit. C and E, Ventral horn motor neurons of riluzole-treated and riluzole plus magnesium-treated rabbits appear grossly normal. D, Most motor neurons from a magnesium-treated rabbit exhibit necrotic changes.
among patients with amyotrophic lateral sclerosis,\textsuperscript{9,11} and it seems devoid of major adverse effects, suggesting that it may be of use in clinical situations involving spinal cord ischemia. Magnesium sulfate (MgSO\textsubscript{4}) is a well-known NMDA receptor antagonist, which is widely used in patients with preeclampsia and which is presently being evaluated in the treatment of acute stroke,\textsuperscript{12,13} suggesting a potential role in the prevention of spinal cord ischemic injury.

The aim of this study was to determine whether MgSO\textsubscript{4} administered before aortic clamping was really capable of preserving the structural integrity of the spinal cord in a setting of severe ischemia, to observe whether riluzole was superior to MgSO\textsubscript{4} in preventing spinal cord ischemic injury, and to demonstrate an eventual additive neuroprotective effect for riluzole and MgSO\textsubscript{4}.

\textbf{MATERIAL AND METHODS}

\textbf{Animal care}

Female New Zealand albino white rabbits weighing 3.5 to 4.5 kg were used in this study. This work was approved by the local Institutional Animal Care Committee. All animals received humane care, in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health (NIH Publication No. 86-23, revised 1985).

\textbf{Anesthesia and monitoring}

Animals fasted for 12 hours and were anesthetized with intramuscular ketamine (50 mg/kg) and xylazine (10 mg/kg). Animals were allowed to breath spontaneously, and general anesthesia was maintained with a mixture of 1.5% halothane and oxygen administered by means of a face cone at a rate of 6 L/min. The core temperature was continuously monitored with a rectal probe inserted 5 cm into the rectum. Rectal temperature was registered from the onset of anesthesia to 1 hour of reperfusion and supported by a heating lamp throughout the procedure. A catheter (24 gauge) was placed in a marginal ear vein, and preoperative Cefazolin (10 mg/kg) was given as a single dose. The heartbeat was monitored throughout the procedure (monitor model 78353B, Hewlett-Packard, Palo Alto, Calif). Catheters (24 gauge) were steriley placed in the central ear artery and right femoral artery to monitor proximal and distal arterial blood pressure, respectively. Percutaneous arterial oxygen saturation (SaO\textsubscript{2}) was continuously monitored.

\textbf{Operative technique}

Animals were placed in the right lateral decubitus position. After sterile preparation, a 10-cm flank incision was made, and the infrarenal aorta was exposed through a retroperitoneal approach. The aorta was isolated from the renal arteries down to the aortic bifurcation. Each animal was given 150 U/kg heparin intravenously 10 minutes before the aorta was occluded. The effects of heparin were not reversed at the end of the procedure. Aortic occlusion was obtained by placing temporary vascular clamps (Biover disposable vessel clip, Arex, France) just below the renal arteries and just above the aortic bifurcation. All animals were subjected to 40 minutes of cross-clamp time. Aortic clamps were removed after 40 minutes, and satisfactory pulsatile distal aortic pressure was verified. The flank was closed in layers. The catheters were removed, and

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
Groups & Control (n = 17) & Group A (n = 17) & Group B (n = 17) & Group C (n = 17) \\
\hline
Weight (kg) & 3.95 ± 0.9 & 3.91 ± 0.9 & 3.98 ± 0.9 & 4.05 ± 0.8 \\
Rectal temperature (°C) & & & & \\
Maxima & 39.2 ± 0.4 & 39.2 ± 0.3 & 39.3 ± 0.4 & 39.3 ± 0.3 \\
Minima & 38.4 ± 0.5 & 38.2 ± 0.4 & 38.2 ± 0.3 & 38.2 ± 0.4 \\
PABP (mm Hg) & & & & \\
Preischemia & 90 ± 6 & 92 ± 8 & 80 ± 5 & 81 ± 3 \\
Ischemia 10 minutes & 92 ± 7 & 92 ± 4 & 83 ± 3 & 88 ± 5 \\
Reperfusion 10 minutes & 79 ± 3 & 82 ± 3 & 77 ± 2 & 78 ± 3 \\
DABP (mm Hg) & 11 ± 1 & 11 ± 1 & 12 ± 2 & 13 ± 1 \\
\hline
\end{tabular}
\caption{Physiologic parameters}
\end{table}

The values are expressed as mean ± SD.
PABP, Proximal arterial blood pressure (central ear artery); DABP, distal arterial blood pressure (femoral artery); Rectal temperature, maxima and minima recorded from the onset of anesthesia to 1 hour reperfusion. There were no significant differences in physiological parameters among the four groups.
the femoral artery was repaired with 8-0 polypropylene suture. Sham-operated animals underwent the same operative conditions, but without aortic clamping. Animals were allowed to recover in their cages, with free access to food and water after 6 hours postoperatively.

**Postoperative period and sacrifice.**

Credé’s maneuver was used twice daily to empty the bladders of the paraplegic animals. Animals were randomly assigned to be humanely killed at 24 hours ($n = 3$ per group), 48 hours ($n = 4$ per group), or 120 hours ($n = 10$ per group) postoperatively. Sham-
operated animals were killed at 24 hours postoperatively. All animals were killed by a lethal injection of pentobarbital (200 mg/ kg). Spinal cords were quickly harvested for histopathologic and immunohistochemistry examinations. The L3-L4 segment was fixed in Hollande Bouin, and the L5-L6 segment was frozen in isopentane on dry ice and stored at -70°C.

Study groups
Each study group represents the animals that survived the entire observation period.

• Sham-operation group (n = 5). The operation was performed with the same conditions, but without aortic clamping.

• Control group (n = 17). The animals received only vehicle (0.9% saline).

• Group A (n = 17). Riluzole (8 mg/kg) was injected intravenously 30 minutes before aortic clamping.

• Group B (n = 17). MgSO₄ (100 mg/kg) was injected intravenously in 10 minutes, 30 minutes before aortic clamping.

• Group C (n = 17). Riluzole (8 mg/kg) and MgSO₄ (100 mg/kg) were injected intravenously in 10 minutes, 30 minutes before aortic clamping.

All animals received a similar volume of solutions: 20 mL for the whole procedure.

Riluzole (Research Biochemicals International, Natick, Mass) was first dissolved in hydrochloric acid 0.1 N, then diluted in distilled water. MgSO₄ was diluted 50% in 0.9% saline before intravenous injection in 10 minutes.

Blood magnesium level measurement
Blood samples (0.5 mL) were drawn 10 minutes before clamping and 10 minutes after the onset of reperfusion in the control group and in group B. Assays of serum-ionized magnesium were performed by means of an automatic clinical analyzer.

Neurologic evaluation
A neurologic assessment was performed in all animals after 3 hours, 6 hours, 24 hours, and then daily, in a blinded fashion. Spinal cord function was graded according to a modified Tarlov scale: 0, no movement; 1, slight movement; 2, sits with assistance; 3, sits alone; 4, weak hop; 5, normal hop.

Tissue preparation
Paraffin sections. Spinal cords (L3-L4 segments) were removed from Hollande Bouin after 24-hour fixation. Specimens were dehydrated in 95% alcohol for 30 minutes, followed by four changes of 100% alcohol for 1 hour each and five changes of toluene for 1 hour each under a vacuum at 37°C. Spinal cords were infiltrated with paraffin and embedded in paraffin at 57°C under vacuum and pressure. Sections were cut on a microtome (Leica) at 7 μm.

Cryostat sections. Spinal cords (L5-L6 segments) were extracted and fresh frozen in isopentane at -45°C. Cryostat coronal sections (10 μm) were mounted on poly-L-lysine-coated slides and tissue postfixed by successive immersion in 0.01 mol/L phosphate buffer (PBS) and 4% paraformaldehyde for 30 minutes. Sections were then dehydrated in ethanol baths (50%, 70%, and 100%), air dried, and stored at -70°C until use.

All animals had their spinal cord studied. For each spinal cord studied, six sections were placed on 3-aminopropylsilane-coated slides, and 10 slides per rabbit were used for spinal cord analysis. The neuropathologist, who was blinded to the experimental conditions and treatment, performed the histopathologic examination with light microscopy.

We used these classifications for spinal cord analysis: normal, normal appearance of motor neurons in gray matter; grade I, less than 33% of motor neurons damaged in gray matter; grade II, 33% to

<table>
<thead>
<tr>
<th>Tarlov score</th>
<th>Sham group (n = 5)</th>
<th>Control group (n = 17)</th>
<th>Group A (n = 17)</th>
<th>Group B (n = 17)</th>
<th>Group C (n = 17)</th>
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Differences between groups were analyzed with Kruskal-Wallis tests, followed by Mann-Whitney U tests when significant. Group A vs control group, P <.01; group B vs control group, P <.01; group C vs control group, P <.01; group A vs group B, P <.01; group A vs group C, not significant; group B vs group C, P <.01.
66% of motor neurons damaged in gray matter; grade III, more than 66% of motor neurons damaged in gray matter.

**Histopathologic examination**

**Luxol fast blue staining method.** Transverse sections were deparaffinized by washing twice in xylene for 5 minutes each and heating at 56°C overnight, and then they were rehydrated through a graded series of alcohols and distilled water. Sections were soaked in 70% alcohol for 10 minutes. They were stained at 57°C for 16 hours in solution A, which contained 1 mg Luxol fast blue (Solvent Blue 38, Sigma) and 5 mL 10% acetic acid in 1000 mL of 95% alcohol. The sections were then rinsed in 70% alcohol for 10 minutes. They were stained at 57°C for 16 hours in solution A, which contained 1 mg Luxol fast blue (Solvent Blue 38, Sigma) and 5 mL 10% acetic acid in 1000 mL of 95% alcohol. The sections were then rinsed in 70% alcohol and distilled water. They were differentiated by dipping the sections singly into 0.05% aqueous Li₂CO₃ solution for a few seconds and then washing them through several changes of 70% alcohol and placing them in distilled water. Sections were then counterstained with neutral red (Sigma). Sections were treated with 0.2% NaHSO₃ for 1 minute and then immersed in 0.1 mol/L acetate buffer for 1 minute. Neutral red staining was performed in solution B, containing 3 vol of 0.05% neutral red stock solution and 2 vol of 0.1 mol/L acetate buffer for 15 minutes at room temperature. Sections were rinsed in distilled water, immersed in copper-sulfate-chrome alum solution for 1 second, and rinsed in distilled water. Sections were then mounted in Aquamount.

**Immunohistochemistry for microtubule-associated protein 2**

Frozen sections (25 µm) were immersed in 0.3% H₂O₂ for 10 minutes, blocked with 5% goat serum (Vector Laboratories) and 3% Triton for 1 hour at room temperature, followed by a rinse in PBS. Then sections were incubated with the primary antibody overnight. The antiserum used for the study of cytoskeletal protein expression was a monoclonal mouse anti-MAP-2 (Clone H M -2, Sigma, diluted 1:500). After the primary incubation and three rinses in PBS, sections were incubated in biotinylated horse anti-mouse IgG (diluted 1:100; Vector Laboratories) for 3 hours. MAP-2 expression was visualized with 3’-diaminobenzidine and nickel chloride (DAB-Ni) staining by using the Vectastain ABC kit (Vector Laboratories). All sections were washed a final time in PBS and distilled water and then mounted with glycerol.

**Statistical analysis**

Statistical analyses of measured physiologic data were performed by means of analysis of variance (ANOVA). All physiologic data are expressed as the mean plus or minus SD. Tarlov scores were analyzed with Kruskal-Wallis tests, followed by Mann-Whitney U tests when significant. Serum magnesium levels were compared by means of one-way ANOVA and Student t tests. Differences were considered statistically significant when the P value was less than .05.

**RESULTS**

**Physiologic parameters.** No significant differences existed in the physiological parameters among the experimental groups (Table I). SaO₂ remained in a normal range in all animals throughout the procedure.

**Neurologic outcome.** All animals survived the entire randomly assigned observation period. All sham-operated animals had a normal postoperative neurologic function, whereas all control rabbits had a Tarlov score of 0 at 24 hours and remained severely paraplegic throughout the observation period. Neurological outcome was significantly better in riluzole-treated animals from groups A and C (P < .01 for group A vs control group; P < .01 for group C vs control group; Tables II, III, and IV). Most animals in

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**Table III.** Neurologic outcome: Tarlov scores at 48 hours.

<table>
<thead>
<tr>
<th>Tarlov score</th>
<th>Control group (n = 14)</th>
<th>Group A (n = 14)</th>
<th>Group B (n = 14)</th>
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Differences between groups were analyzed with Kruskal-Wallis tests, followed by Mann-Whitney U tests when significant. Group A vs control group, P < .01; group B vs control group, P < .01; group C vs control group, P < .01; group A vs group B, P < .01; group A vs group C, not significant; group B vs group C, P < .01.
those groups had either a normal function or a mild-to-moderate deficit. The addition of MgSO4 to riluzole did not further improve neurological status ($P = \text{not significant for group A vs group C}$). No riluzole-treated rabbit had deterioration of neurologic status between 24 and 120 hours, whereas two rabbits in group B had a worsening of their neurologic score ($P < .01$ for group A vs group B and $P < .01$ for group C vs group B, at any time considered).

**Blood magnesium levels.** Blood magnesium levels before clamping were $1.68 \pm 0.26$ mEq/L in the control group and $6.47 \pm 1.3$ mEq/L in group B (range, 4.70 to 8.90 mEq/L; $P < .01$ for group B vs control group). During reperfusion, the values were $1.81 \pm 0.15$ mEq/L and $3.89 \pm 0.48$ mEq/L in the control group and group B, respectively ($P < .01$ for group B vs control group). We did not observe any difference in neurologic outcome between rabbits with a peak serum magnesium level higher than 7 mEq/L and those with a peak serum magnesium level lower than 7 mEq/L.

**Histopathologic examination.** Luxol fast blue staining was used as a means of analyzing neuronal cell death. The extent of ischemic damage was grossly proportional to the Tarlov score. Ischemic damage was observed almost exclusively in gray matter, which contained typically necrotic neurons in ventral and dorsal horns and in the intermediate zone. Sham-operated animals had normal spinal cords. Rabbles with a Tarlov score of 2 or 1 usually had more extensive necrotic damage in all ventral gray matter areas (grade III lesions), which also extended to the dorsal horns. Most riluzole-treated animals (groups A and C) had either no or few signs of neuronal damage: 65% of rabbits had normal spinal cords and only 15% of rabbits had grade III lesions (Table V). Magnesium-treated animals (group B) usually had moderate-to-severe gray matter involvement, with less than 20% of rabbits having normal spinal cords and 59% of rabbits having grade III lesions. All control animals had a very severe involvement, with global necrosis of gray matter: 100% of rabbits had severe grade III lesions. In most severe cases, white matter surrounding the gray matter was also involved.

**Cytoskeletal proteolysis/microtubule-associated protein-2 immunohistochemistry.** The cytoskeletal MAP-2 is involved in maintaining neuronal structural integrity. It is extremely sensitive to ischemia, and MAP-2 immunostaining has been demonstrated to be a sensitive, accurate, and early marker of ischemic injury after cerebral ischemia.$^{15}$ MAP-2 immunoreactivity was expressed exclusively in the gray matter and grossly paralleled histopathologic findings in most animals. MAP-2 was strongly expressed in neuronal perikaryon and dendrites of sham-operated animals and in animals with a Tarlov score of 5. All control animals had a complete loss of MAP-2 immunoreactivity, as early as 24 hours after ischemia. Most riluzole-treated animals (groups A and C) had either a grossly normal MAP-2 immunoreactivity or focal decrease of immunoreactivity in central gray matter and ventral horns. In most riluzole-treated animals, MAP-2 immunoreactivity was completely preserved for as long as 5 days of reperfusion, attesting to the optimal protection against ischemic cytoskeletal breakdown.

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### Table IV. Neurologic outcome: Tarlov scores at 120 hours

<table>
<thead>
<tr>
<th>Tarlov score</th>
<th>Control group (n = 10)</th>
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Differences between groups were analyzed with Kruskal-Wallis tests, followed by Mann-Whitney U tests when significant. Group A vs control group, $P < .01$; group B vs control group, $P < .01$; group C vs control group, $P < .01$; group A vs group B, $P < .01$; group A vs group C, not significant; group B vs group C, $P < .01$. 

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nesium-treated rabbits (group B) had lost more than 66% of MAP-2 immunostaining in ventral horns and central gray matter, attesting to the incomplete protection against ischemic cytoskeletal breakdown.

**DISCUSSION**

Paraplegia remains a serious complication of thoracic or thoracoabdominal aortic surgery. Postoperative neurologic deficits occur in 3.7% to 21% of patients, depending on the presence of acute dissection, the level of aortic repair or replacement, the extension of aortic replacement, the presence of critical intercostal arteries within the replaced aortic segment, and the duration of aortic clamping.1,2

Because periods of spinal cord ischemia are often ineluctable in major cases, pharmacologic interventions have been attempted by some investigators.16 Unfortunately, no drug consistently proved effective in clinical practice.

Recent experimental and clinical studies confirmed the role played by excitotoxic mechanisms in the pathophysiology of spinal cord ischemic injury after the repair of thoracoabdominal aortic aneurysms.3,17 Since the experimental work of Choi and colleagues,18 evidence has accumulated that the excitatory amino acids (EAAs), particularly glutamate, the primary neurotransmitter in the spinal cord, have potent neurotoxic activity during conditions of depleted cellular energy such as ischemia, when the synaptic reuptake of EAAs, a highly energy-dependent process, becomes compromised.4

Excitotoxicity is a delayed phenomenon, extending for several hours after the initiation of ischemia.4 Thus, pharmacological interventions aimed at minimizing spinal cord ischemic injury may be started before, during, and also after aortic cross-clamping.

NMDA and non-NMDA receptor antagonists reliably afford protection against ischemic injury in the brain. These compounds have been shown to be neuroprotective in several animal models of cerebral or spinal cord ischemia.3-6 However, these compounds can cause widespread central nervous system depression, learning impairment, and pathological changes in defined populations of neurons.7,19

In addition, recent studies suggested that glutamate excitotoxicity in the spinal cord is mediated by NMDA, but also non-NMDA receptors, accounting for incomplete neuroprotection by NMDA or non-NMDA receptor antagonists alone.3,6,20

In our study, intravenous MgSO4 resulted in significant spinal cord protection. These results are in accordance with earlier studies on the same model.21,22 The neuroprotective effects of magnesium ions have been used for many years, so MgSO4 has become the standard therapy for preeclampsia in the United States.12 The dose of magnesium used in this study (100 mg/ kg) corresponds to the loading dose used in patients with preeclampsia, and blood magnesium levels in our study correspond to those reported in humans after an identical loading dose.12 We did not observe adverse effects, such as respiratory depression, severe hypotension, or major electrocardiographic changes, with MgSO4 concentrations as high as 8.90 mEq/L. Magnesium ions cross the intact blood-brain barrier, so intravenous MgSO4 significantly raises cerebrospinal fluid (CSF) magnesium concentration.12 It is probable that higher CSF concentrations may be achieved when blood-brain barrier integrity is compromised by ischemia.13 In our study, MgSO4 (100 mg/ kg) given as a single injection before aortic clamping afforded significant neuroprotection. However, intravenous MgSO4 afforded incomplete protection, because most animals had only a partial recovery.

Histopathological and MAP-2 immunohistochemistry examinations confirmed that MgSO4 incompletely prevented neuronal necrosis and cytoskeletal proteolysis. Recently, Simpson et al23 reported that intrathecal MgSO4 (3 mg/ kg) could dramatically improve neurological status after 45 minutes of thoracic aortic clamping in dogs. Differences in peak CSF magnesium concentration, between our animals and those reported by Simpson et al,27 may account for the difference in the animals' neurological outcome between the two studies. Thus, increasing the loading dose of MgSO4 may be required to achieve superior CSF peak concentrations and subsequent neuroprotection. Further studies are warranted.

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Sham group (n = 5)</th>
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to observe whether a superior loading dose (ie, 150 mg/kg) may provide superior neuroprotection, without adverse effects. Magnesium’s mechanism of neuroprotection is probably not univocal. First, magnesium is a well-known noncompetitive NMDA receptors blocker and endogenous Ca2+ channel blocker. Second, magnesium may cause vasodilation of vessels supplying the spinal cord by stimulating endothelial prostacyclin release. Third, magnesium may prevent thrombosis of critical segmental vessels by inhibiting platelet reactivity. Fourth, magnesium may decrease endothelial and neuronal reperfusion injuries by directly inhibiting lipid peroxidation and by preventing glutathione depletion. Fifth, magnesium may regulate adenosine triphosphatase concentrations and regenerate adenosine triphosphate after ischemia and reperfusion. Magnesium is easily and widely available, inexpensive, and has been used for years in clinical practice for the treatment of myocardial infarction, arrhythmia, and stroke, without adverse effects.

Riluzole (2-amino-6-trifluoromethoxybenzothiazole) is a neuroprotective drug that blocks glutamatergic neurotransmission in the central nervous system. The exact molecular target of riluzole is not known. However, experimental studies suggested that riluzole has a complex mechanism of action, including a direct, but noncompetitive, blockade of ionotropic EAAs receptors, inhibition of presynaptic glutamate release, inactivation of voltage-dependent calcium and sodium channels, and stimulation of a G-protein-dependent signal transduction pathway. Recently, riluzole was demonstrated to activate a new class of background 2 P domain K+ channels that are highly expressed in the spinal cord, and that may play a role during neuronal ischemia. It is not known whether these mechanisms are really independent of one another. However, it is likely that the principal effects act synergistically at glutamatergic synapses to diminish neurotransmission. In vivo, riluzole has demonstrated neuroprotective action in several models of neuronal injury known to involve excitotoxic mechanisms, including focal or global cerebral ischemia models, posttraumatic spinal cord injury models, and spinal cord ischemia models. In addition, riluzole has other potentially beneficial effects on the central nervous system, such as sedative and anesthetic properties, and a potent anticonvulsant activity. Although high doses of riluzole slightly modify the cardiac action potential in vitro, this drug does not have appreciable cardiovascular effects in vivo, particularly on arterial blood pressure, heart rate, or electrocardiographic parameters. Riluzole is currently given to patients with amyotrophic lateral sclerosis, and it seems devoid of major adverse effects. The dose of riluzole used in this study (8 mg/kg) is the dose that has been reported to be neuroprotective in several studies. This study confirms that a single dose of riluzole given before aortic clamping may result in significant spinal cord protection, even in a setting of severe ischemia. The addition of MgSO4 to riluzole did not further improve neurologic outcome, compared with riluzole given alone, suggesting that no synergistic effect exists for those drugs when given in combination. This association resulted in a better neurological status than magnesium given alone (P < .01), and the neurological outcome was better in riluzole-treated rabbits than in magnesium-treated rabbits (P < .01). These results suggest that riluzole may be superior to MgSO4 (100 mg/kg) for the prevention of spinal cord ischemia injury. The tolerance of the spinal cord to ischemia was demonstrated by means of the analysis of postoperative neurological status to be significantly increased by riluzole and, to a lesser extent, by MgSO4. In addition, we did not observe a secondary worsening of motor deficits between 24 and 120 hours in riluzole-treated animals, as we did in magnesium-treated animals. The worsening of neurological status and delayed paraplegia have been reported in this model by other investigators. Histopathologic and MAP-2 immunohistochemistry examinations confirmed that riluzole prevented neuronal necrosis and ischemia-induced cytoskeletal proteolysis. Riluzole was particularly effective in preventing motor neuron injury, which is important because motor neurons are usually extremely sensitive to excitotoxicity and constitute one of the main neuronal components that is damaged after spinal cord ischemia.

The model used in this study is highly reproducible. Severe spinal cord ischemia is induced, which usually results in immediate and definitive paraplegia in 100% of animals. The potential limitations of this study include the use of halothane and ketamine, which have been reported to be neuroprotective. In addition, we recorded an approximately 1°C decrease in rectal temperature of all animals between the onset and the end of the procedure. All those factors may have influenced the level of spinal cord ischemia. However, the use of halothane and ketamine, and the mild decrease in body temperature of animals cannot account for the neuroprotective effects observed in riluzole-treated and magnesium-treated animals, because control animals that were operated on with the same condi-
tions had a significantly worse neurological outcome and definitive paraplegia. We did not measure blood glucose concentration in any animal. It has been widely demonstrated that blood glucose concentration affects ischemic spinal cord injury.38 However, all rabbits in this study were fasted for 12 hours before surgery, and none received glucose solution preoperatively, making differences in blood glucose concentrations among experimental groups highly improbable.

CONCLUSION

The use of riluzole and MgSO₄ resulted in no major adverse effects and afforded significant neuroprotection in this rabbit model of severe spinal cord ischemia. Riluzole was clearly superior to MgSO₄ (100 mg/kg) in preventing spinal cord ischemic injury and related neurologic deficits. Considering the beneficial use of both drugs in several models of neuronal ischemia and the absence of reported adverse effects in clinical studies involving these drugs, our results support the evaluation of MgSO₄ and riluzole for spinal cord protection during high-risk thoracoabdominal aortic surgery.

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REFERENCES


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