 

# *Neuroprotective Effects of Riluzole and Ketamine during Transient Spinal Cord Ischemia in the Rabbit*

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***Background:*** **Massive release of central excitatory neurotransmitters is an important initial step in ischemic neuronal injury, and modification of this process may provide neuroprotection. We studied the protective effects of the voltage-dependent sodium channel antagonist riluzole and the *N*-methyl-D-aspartate receptor antagonist ketamine on hind limb motor function and histopathologic outcome in an experimental model of spinal cord ischemia.**

***Methods:*** **Temporary spinal cord ischemia was induced by 29 min of infrarenal balloon occlusion of the aorta in 60 anesthetized New Zealand white rabbits. Animals were randomly assigned to one of four treatment groups (n = 15 each): group C, saline (control); group R, riluzole, 8 mg/kg intravenously; group K, ketamine, 55 mg/kg intravenously; group RK, riluzole and ketamine. After reperfusion, riluzole treatment was continued with intraperitoneal infusions. Normothermia (38°C) was maintained during ischemia, and rectal temperature was assessed before and after intraperitoneal infusions. Neurologic function, according to Tarlov's criteria, was evaluated every 24 h, and infarction volume and the number of eosinophilic neurons and viable motoneurons in the lumbosacral spinal cord was evaluated after 72 h.**

***Results:*** **Neurologic outcome was better in groups R and RK than in groups C and K. All animals in group C (100%) and all animals but one in group K (93%) were paraplegic 72 h after the ischemic insult *versus* 53% in group R and 67% in group RK ($P < 0.01$ each). More viable motoneurons were present in groups R and RK than in controls ($P < 0.05$).**

***Conclusions:*** **The data indicate that treatment with riluzole can increase the tolerance of spinal cord motoneurons to a period of normothermic ischemia. Intraischemic ketamine did not provide neuroprotection in this model. (Key words: Excitatory amino acids; glutamate; infarction volume.)**

SELECTIVE blocking of receptors involved in excitatory neurotransmission can protect neuronal populations against normothermic ischemic injury *in vivo*.[1–3] In particular, glutamate release and its postsynaptic action exerted *via* the *N*-methyl-D-aspartate (NMDA) receptor are key features in the triggering of processes that ultimately result in ischemic neuronal damage.[4,5] The sodium channel inhibitor riluzole (2-amino-6-trifluoromethoxybenzothiazole) has been reported to decrease $K^+$-evoked[6] and spontaneous[7] glutamate release and seems to possess noncompetitive NMDA receptor antagonist properties.[8] Riluzole is used clinically for the treatment of patients with amyotrophic lateral sclerosis, a neurodegenerative disease that involves compromised glutamate handling by motoneurons.[9] Riluzole was found to be neuroprotective in behavioral and histologic models of cerebral ischemia.[10,11] Recent evidence indicates that intraischemic treatment with riluzole improves early neurologic outcome (24 h) and may alter the pattern of ischemia-induced apoptosis and necrosis after transient spinal cord ischemia (SCI).[12]

The anesthetic agent ketamine, which has known properties of a noncompetitive NMDA receptor antagonist, reduces neuronal damage from incomplete cerebral ischemia[1,13] and has been used as part of the anesthetic technique in some surgical procedures that carry a risk of paraplegia.[14,15] Although riluzole influences glutamate release at the level of the nerve terminals, additional postsynaptic blockade by ketamine might result in a synergistic protective effect on spinal cord neurons during a transient ischemic period. The purpose of this study was to investigate the effects of riluzole and ketamine, alone and in combination, on neurologic and histopathologic outcome after 72 h in a rabbit model of temporary SCI.

## Materials and Methods

Animal care and all procedures were performed in compliance with the national guidelines for care of laboratory animals in The Netherlands. The study protocol was approved by the Animal Research Committee of the Academic Hospital at the University of Amsterdam, The Netherlands. Sixty New Zealand white rabbits weighing 3.4 ± 0.3 kg (mean ± SD) were used in this study.

### *Anesthesia and Monitoring*

Anesthesia was induced by inhalation of a mixture of 50% $O_2$ in $N_2O$ followed by isoflurane (3%) by mask; after tracheal intubation, anesthesia was maintained with a combination of isoflurane (1.5%) and intravenous sufentanil (5 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Cefamandol (100 mg) was given before the incision was made. End-tidal carbon dioxide was measured by a mainstream capnograph

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(Hewlett-Packard, Boeblingen, Germany), and arterial carbon dioxide tension was maintained within 35–40 mmHg. The initial tidal volume was 20 ml/kg, and the respiratory rate was 40 breaths/min. Rectal and paraspinal muscle temperatures were monitored and kept at 38°C (normothermia) by means of a heating lamp. Paraspinal muscle temperature was measured using a needle probe (subcutaneous temperature sensor, Monatherm Inc., St. Louis, MO), connected to a Monatherm model 6510 (Mallinkrodt Medical, Inc., St. Louis, MO). The electrocardiogram was recorded with pediatric surface electrodes, and the mean arterial blood pressure (MAP) was measured with a double-lumen wedge pressure balloon catheter placed in the abdominal aorta. If MAP decreased to lower than 60 mmHg during reperfusion, animals were given 2.5 mg ephedrine intravenously. Before, during, and after aortic occlusion, 1-ml arterial blood samples were drawn for measurement of blood gases and hematocrit.

*Operative Technique*

Under sterile conditions, a right femoral arteriotomy was performed 3–4 cm distal to the inguinal ligament. A 5-French double-lumen wedge pressure balloon catheter (AI 07025, Arrow International Inc., Reading, PA) was advanced 15 cm into the femoral artery. This resulted in balloon location 0.5–1.5 cm distal to the left renal artery in the abdominal aorta in a previous study.[16] Before catheter insertion, heparin 500 IU was administered intravenously, followed by 500 IU every 30 min thereafter until catheter removal. The balloon was inflated until loss of pulsatile distal aortic pressure, as measured at the distal orifice of the catheter. The duration of aortic occlusion was 29 min, based on an expected 80–85% paraplegia rate as observed in halothane-anesthetized animals.[17] After balloon removal, the wound was closed. An 8-French, 50-cm-long catheter (nasal probe-PVC; Vygon Laboratories, Ecouen, France) was placed from a subcutaneous port in the neck into the intraperitoneal cavity. At this point, animals were allowed to recover. The period between reperfusion and extubation was kept constant (80 min) to account for a possible confounding neuroprotective effect of a longer duration of anesthesia in animals treated with one or both of the study agents. Inhalation of isoflurane was stopped just before the end of the 80-min period. Animals were replaced when postoperative systemic complications necessitated premature termination of the experiment.

*Drug Administration*

Animals were randomly assigned to one of four treatment groups (n = 15 in each group): control (C), riluzole (R), ketamine (K), or riluzole plus ketamine (RK). Riluzole (Rhône-Poulenc Rorer, Antony, France) was dissolved in a solvent containing 0.9% NaCl and 4% HCl 0.1 M (pH 3; maximal volume, 10 ml/kg). Ketamine was dissolved in 0.9% NaCl (pH 7.5; maximal volume, 3 ml/kg). Fifteen min before aortic occlusion, the animals received an intravenous injection of solvent (group C and K) or riluzole 8.0 mg/kg (group R and RK). Subsequently, a second intravenous injection containing ketamine 10 mg/kg was given to animals in groups K and RK. Ten min after reperfusion, ketamine 1.5 mg $\cdot$ kg$^{-1}$ $\cdot$ min$^{-1}$ was given intravenously for 30 min (total dose = 45 mg/kg) to animals in groups K and RK. After emergence from anesthesia, the animals received solvent (groups C and K) or riluzole 8 mg/kg (groups R and RK) in the same dosage twice daily for 3 days *via* the intraperitoneal catheter. Rectal temperature was measured just before and 1 h after each intraperitoneal infusion.

*Neurologic Evaluation*

Twenty-four, 48, and 72 h after the ischemic insult, the neurologic status of each animal was assessed by an observer blinded to the treatment allocation. Assessment was made according to Tarlov's criteria, which consists of a five-point grading scale: 0, paraplegic with no lower extremity function; 1, poor lower-extremity function, weak antigravity movement only; 2, some lower-extremity motor function with good antigravity strength but inability to draw legs under body or hop; 3, ability to draw legs under body and hop but not normally; and 4, normal motor function. In paraplegic animals, bladder contents were expressed manually as required.

*Spinal Cord Pathology*

After final scoring of neurologic function at 72 h, the animals were anesthetized with ketamine (50 mg/kg intramuscularly), xylazine (10 mg/kg intramuscularly), and isoflurane (1 minimum alveolar concentration) in a mixture of 50% $O_2$ in $N_2O$. After administration of heparin (2,500 IU), the animals were sacrificed using pentobarbital (100 mg intravenously) and perfusion fixated with formalin 3.6%. Each lumbosacral spinal cord was removed *en bloc* and immersed in formalin for at least 10 days. The whole lumbosacral portion of the spinal cord was sampled systematically.[18] Twelve equidistant transverse slices, each 1 mm thick, were dissected and embedded in paraffin. From each paraffin block, randomly selected 4-$\mu$m-thick sections were cut and stained with hematoxylin and eosin. One section from each block was evaluated by an observer blinded to the treatment condition as described below.

*Infarction Volume*

At low magnification, all of the sections were digitized and the areas of total gray matter and infarcted gray matter were measured interactively using image analysis software (Qwin, Leica, Cambridge, United Kingdom). The areas (in square millimeters) were then integrated with the known distance between each transverse level to provide an estimate of the infarction volume of the

**Table 1. Physiologic Variables**

| Group | Proximal MAP (mmHg) | Distal MAP (mmHg) | HR (beats/min) | *p*H | $Pa_{O_2}$ (mmHg) | $Pa_{CO_2}$ (mmHg) | Hematocrit |
|---|---|---|---|---|---|---|---|
| Control | | | | | | | |
| Preischemia | 70 ± 11 | | 210 ± 44 | 7.49 ± 0.09 | 173 ± 38 | 32.0 ± 5.4 | 33.5 ± 8.3 |
| Ischemia | 79 ± 12 | 14 ± 4 | 204 ± 64 | 7.51 ± 0.05 | 188 ± 8 | 33.5 ± 2.1 | 34.1 ± 7.4 |
| Reperfusion | 65 ± 11 | | 217 ± 47 | 7.46 ± 0.07 | 163 ± 41 | 29.7 ± 4.5 | 35.8 ± 5.3 |
| Riluzole | | | | | | | |
| Preischemia | 62 ± 11 | | 177 ± 22 | 7.49 ± 0.07 | 199 ± 47 | 32.7 ± 6.1 | 36.0 ± 3.9 |
| Ischemia | 88 ± 30 | 14 ± 6 | 187 ± 53 | 7.50 ± 0.03 | 176 ± 36 | 29.7 ± 3.4 | 35.2 ± 3.8 |
| Reperfusion | 66 ± 11 | | 208 ± 47 | 7.42 ± 0.04 | 166 ± 41 | 33.2 ± 4.2 | 35.7 ± 2.7 |
| Ketamine | | | | | | | |
| Preischemia | 75 ± 29 | | 183 ± 51 | 7.52 ± 0.06 | 172 ± 29 | 35.8 ± 5.5 | 35.0 ± 8.7 |
| Ischemia | 88 ± 27 | 14 ± 7 | 204 ± 60 | 7.41 ± 0.08 | 160 ± 41 | 34.4 ± 6.7 | 34.4 ± 8.5 |
| Reperfusion | 56 ± 19 | | 218 ± 42 | 7.44 ± 0.05 | 178 ± 6 | 28.3 ± 3.3 | 32.3 ± 6.2 |
| Riluzole–ketamine | | | | | | | |
| Preischemia | 62 ± 19 | | 212 ± 39 | 7.47 ± 0.11 | 186 ± 32 | 34.4 ± 7.2 | 31.3 ± 6.3 |
| Ischemia | 77 ± 18 | 20 ± 7 | 203 ± 50 | 7.45 ± 0.07 | 162 ± 45 | 29.2 ± 5.1 | 33.0 ± 6.5 |
| Reperfusion | 64 ± 19 | | 216 ± 46 | 7.43 ± 0.06 | 164 ± 47 | 34.2 ± 7.8 | 31.4 ± 1.9 |

All values are mean ± SD.

MAP = mean arterial pressure; HR = heart rate; $Pa_{O_2}$ = arterial oxygen tension; $Pa_{CO_2}$ = arterial carbon dioxide tension; preischemia = period before occlusion; ischemia = occlusion; reperfusion = period after occlusion.



spinal cord. In each animal, the extent of infarction was expressed as the percentage of necrotic tissue of the total gray matter volume. To further specify the localization of infarctions, gray matter area was separated into dorsal, intermediate, and ventral zones by dividing the dorsoventral axis of gray matter into three equal parts.

*Selective Neuronal Necrosis*

To quantify selective necrosis, eosinophilic neurons were counted in every section of the spinal cord using light microscopy (Leica). Individual counts were added to provide an aggregate of eosinophilic neurons for all 12 sections. The effective magnification was 100×.

*Ventral Horn Motoneurons*

The total number of apparently viable ventral horn ($\alpha$) motoneurons was determined in each section. Morphologic viability was defined according to the following criteria: fine granular cytoplasm with basophilic stippling (presence of Nissl substance),[19] prominent nucleoli, and a soma diameter of 30–60 $\mu$m. Results were expressed as aggregates of 12 counts for each animal, one count being the total number of motoneurons for one section.

*Statistical Analysis and Presentation of Results*

Power analysis was used to calculate the minimum group size that allowed for detection of significant differences in neurologic outcome among the treatment groups. We wished to have sufficient power ($1\text{-}\beta = 0.8$, $\alpha = 0.05$) to be able to detect a 50% reduction of the paraplegia rate in the riluzole group, assuming a 85% event rate in the reference group; this required a group size of 15. Hemodynamic data, blood gases, and temperatures are expressed as means ± SD. Tarlov scores are presented as medians and 10th to 90th percentiles. Infarction volumes and neuron counts are expressed as medians and interquartile ranges. The physiologic variables were analyzed using a one-way analysis of variance, and when significant differences were identified, Student *t* tests for intergroup comparisons with appropriate correction for multiple comparisons were performed. Rectal temperatures before and after intraperitoneal infusions were analyzed using analysis of variance for repeated measurements. Comparison of the overall incidence of neurologic deficits (Tarlov < 4), incidence of paraplegia (Tarlov = 0), and paraparesis (Tarlov = 1, 2, or 3) was carried out using the Fisher exact test. To test for the possibility that treatment could influence the pattern of improvement or worsening of neurologic function over time, a repeated measurements test for nonparametric data (Friedman test) was performed using riluzole-treated *versus* non–riluzole-treated as the grouping variable. To examine the correlation between neurologic function and infarction volumes, neurologic scores were classified as paraplegia, paraparesis, or normal motor function. Infarction volumes, neuron counts, and Tarlov scores were analyzed *via* the Kruskall-Wallis test, followed by the Mann–Whitney U test when indicated. A $P$ value < 0.05 was considered significant.

## Results

There were no differences in average weight of the animals among the treatment groups. Table 1 summarizes the hemodynamic and blood gas data before, during, and after aortic occlusion. No differences in MAP, heart rate, pH, arterial oxygen tension, arterial carbon dioxide tension, or hematocrit were observed among the four groups. Drug treatment decreased MAP in several

**Table 2. Paravertebral and Rectal Temperatures**

| Operation | | | | Postoperative Period | | |
|---|---|---|---|---|---|---|
| Treatment Group | Intravenous Administration | Paravertebral Muscle Temperature (°C) | Rectal Temperature (°C) | Intraperitoneal Administration | Before Infusion (°C) | 1 h after Infusion (°C) |
| Control | NaCl | 37.8 ± 0.5 | 37.8 ± 0.6 | NaCl | 39.0 ± 0.7 | 39.0 ± 0.8 |
| Riluzole | Riluzole | 38.0 ± 0.5 | 37.9 ± 0.6 | Riluzole | 39.1 ± 0.6 | 38.9 ± 0.5 |
| Ketamine | Ketamine | 37.7 ± 0.8 | 37.7 ± 0.6 | NaCl | 38.9 ± 0.9 | 39.1 ± 0.8 |
| Riluzole + ketamine | Riluzole + ketamine | 37.8 ± 0.6 | 37.8 ± 0.5 | Riluzole | 38.9 ± 0.7 | 38.7 ± 0.7 |

Paravertebral and rectal temperatures were recorded during operation; rectal temperatures were recorded immediately before and 1 h after intraperitoneal infusion of the study drug. Data are expressed as mean ± SD.

animals; ephedrine (single 2.5-mg injection before occlusion) was necessary to maintain MAP in three rabbits in group C, three in group R, seven in group RK, and eight in group K. Normothermia (38.0°C) was maintained during the operation, and no differences in paraspinal or rectal temperatures were observed among the four groups (table 2). Temperatures before and after intraperitoneal injection were similar in all groups (table 2). Postmortem examination of the abdominal wall and organs did not reveal any reactive serosal changes as a

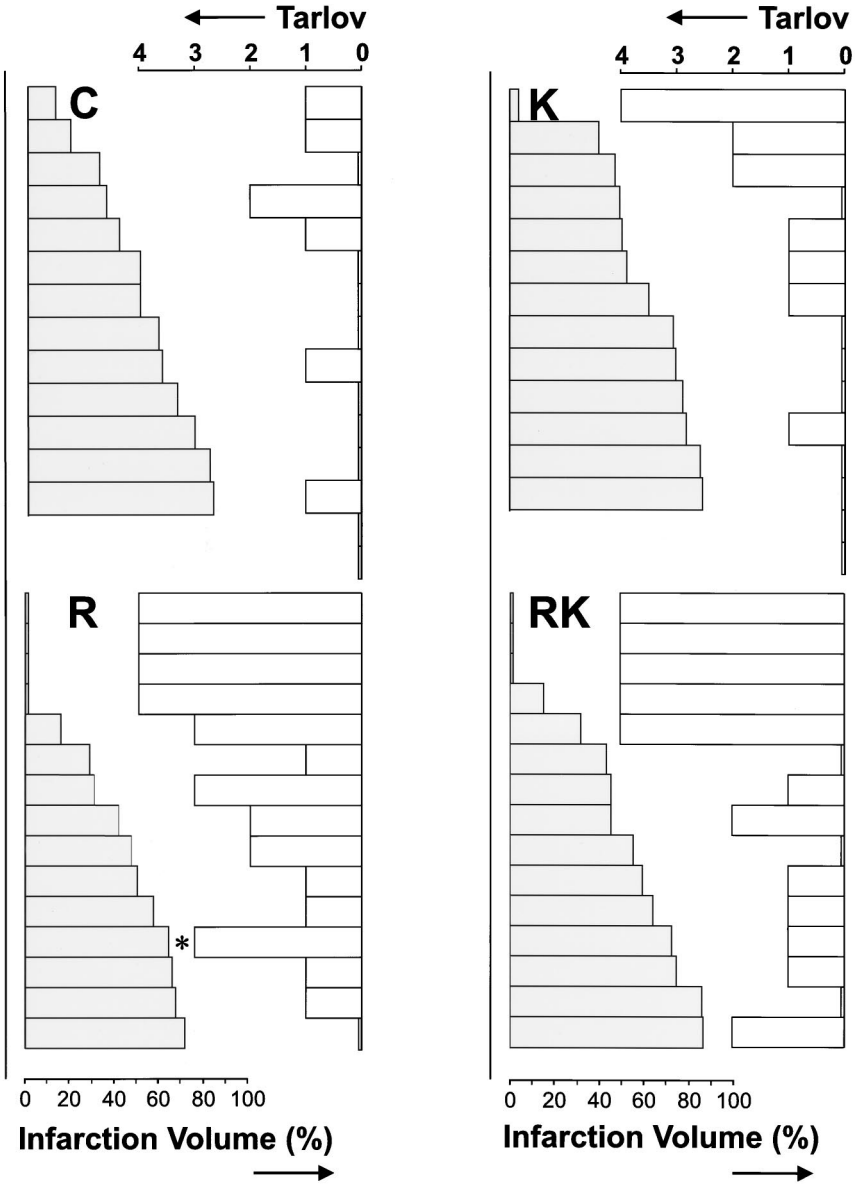


**Fig. 1. Histograms of neurologic (Tarlov) score and relative infarction volume per animal in the four treatment groups. Infarction volume is shown at the left of each diagram (abscissa at bottom), and neurologic score is shown at the right of each diagram (abscissa at top). Neurologic score: 0, paraplegic with no lower-extremity function; 1, poor lower-extremity function, weak antigravity movement only; 2, some lower-extremity motor function with good antigravity strength but inability to draw legs under body or hop; 3, ability to draw leg under body and hop but not normally; 4, normal motor function. C = control; K = ketamine; R = riluzole; RK = riluzole plus ketamine. The infarcted gray matter of this animal is shown in figure 3B (*).**

**Table 3. Neurologic Damage 24, 48, and 72 h after Spinal Cord Ischemia in the Four Treatment Groups**

| Treatment Group | Incidence Complete Paraplegia (Tarlov = 0) | | | Incidence Paraparesis (Tarlov = 1, 2, or 3) | | |
|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| Control | 8 | 8 | 9 | 7 | 7 | 6 |
| Riluzole | 4 | 1* | 1* | 7 | 10 | 10 |
| Ketamine | 6 | 9 | 8 | 8 | 5 | 6 |
| Riluzole + ketamine | 5 | 3 | 3† | 5 | 8 | 7 |

Each group consists of 15 animals. Tarlov score: 0 = paraplegic with no lower-extremity function; 1 = poor lower-extremity function, weak antigravity movement only; 2 = some lower-extremity motor function with good antigravity strength but inability to draw legs under body or hop; 3 = ability to draw leg under body and hop, but not normally; 4 = normal motor function.
* $P < 0.01$, † $P < 0.05$ as compared with controls.

result of the low pH of the intraperitoneal infusion fluids. Seven animals were replaced during the course of the study: four animals were sacrificed intraoperatively (insufficient duration of SCI because of technical failure of the catheter resulting in premature balloon deflation [n = 3]; expiration of the riluzole solution detected after intravenous administration [n = 1]). Three rabbits were euthanized because of postoperative systemic complications. One of these animals (group RK; Tarlov score = 3) had pneumonia, and two animals (one in group C and one in group R; Tarlov scores = 0 and 1, respectively) were euthanized because of stress associated with the neurologic deficit.

*Neurologic Outcome*

Riluzole-treated animals in groups R and RK exhibited a significant reduction in overall neurologic deficits, a decrease in the incidence of paraplegia, and improved Tarlov scores compared with controls ($P < 0.05$, $P < 0.001$, and $P < 0.01$ respectively; fig. 1; table 3).

The median Tarlov scores (interquartile ranges) 72 h after induction of ischemia were 0 (0–1) in group C, 2 (1–3.8) in group R) 0 (0–1) in group K, and 1 (1–4) in group RK. One animal in group K and none in group C had normal hind limb function. In contrast, seven animals in group R and five in group RK were able to hop. Most of the neurologic deficits were present 1 day after SCI. In 26% of the animals, the Tarlov score changed after the initial score at 24 h after reperfusion (table 3). There was no significant effect on recovery pattern over time—neither worsening of neurologic function in the nonriluzole group nor improvement in the riluzole group.

*Histopathology*

In the animals with histologic injury, the infarctions typically affected intermediate gray matter and the anterior horn; infarction volumes were 46% and 44% of gray matter total volume, respectively. Damage to the dorsal horn was rare (10% of gray matter total volume). Four animals were excluded from histopathologic analysis because of damaged or insufficient material (2 in group C and 2 in group K).

The histopathologic results are shown in table 4. In the ventral horn of riluzole-treated animals, more viable motoneurons were present compared with controls (fig. 2; table 4; $P < 0.05$). The total number of eosinophilic neurons was not different among the groups.

Figure 1 shows individual scores of neurologic function (Tarlov scores) and infarction volume for all animals. Ten animals had normal motor function (Tarlov = 4), of which nine had received riluzole. These animals showed minimal histopathologic injury. Infarction volumes were not significantly different among the treatment groups. There was no difference in relative or absolute infarction volume after subdivision of the total infarcted area into ventral, intermediate, and dorsal zones. To investigate the correlation between histopathologic injury and neurologic function to a further extent, the Tarlov score was recoded as paraplegia (Tarlov = 0; n = 17), paraparesis (Tarlov = 1, 2, or 3; n = 29), and normal motor function (Tarlov = 4; n = 10).

A significant difference in infarction volumes was shown among these three groups (table 5). Infarction volumes (mean ± SD) of paraplegic animals were less in the ventral (56.4 ± 25.1 $mm^3$), intermediate (63.5 ± 23.5 $mm^3$), and dorsal zones (16.8 ± 12.5 $mm^3$) compared with neurologically normal animals (4.3 ± 8.0, 6.2 ± 13.6, and 0.6 ± 1.3 $mm^3$ in the respective zones; $P < 0.0001$). Infarction volumes in the paraparetic group were less in the intermediate (20.4 ± 19.3 $mm^3$) and dorsal zones (10.3 ± 8.4 $mm^3$; $P < 0.05$), but not in the ventral zone (44.4 ± 17.4 $mm^3$; $P = 0.06$), compared with paraplegic animals. Infarction volumes were more extensive in the paraparetic group than in the normal motor function group ($P < 0.0001$).

## Discussion

Periischemic riluzole improved neurologic outcome 72 h after temporary SCI. This effect was not dependent on drug-induced mild hypothermia. These results are consistent with studies in which riluzole improved memory deficit[10] and reduced neuronal injury[11] in gerbils that underwent transient bilateral carotid occlusion.

In the current study, we produced SCI in the rabbit by infrarenal aortic occlusion. This is a highly reproducible model for the production of SCI injury, because this animal has a segmental blood supply to the spinal cord with poor collateral flow between the segments. In general, a clear relation exists between the occlusion period and the histopathologic damage and clinical function.[20] This makes it a reliable model for assessment of putative neuroprotective pharmacologic agents.

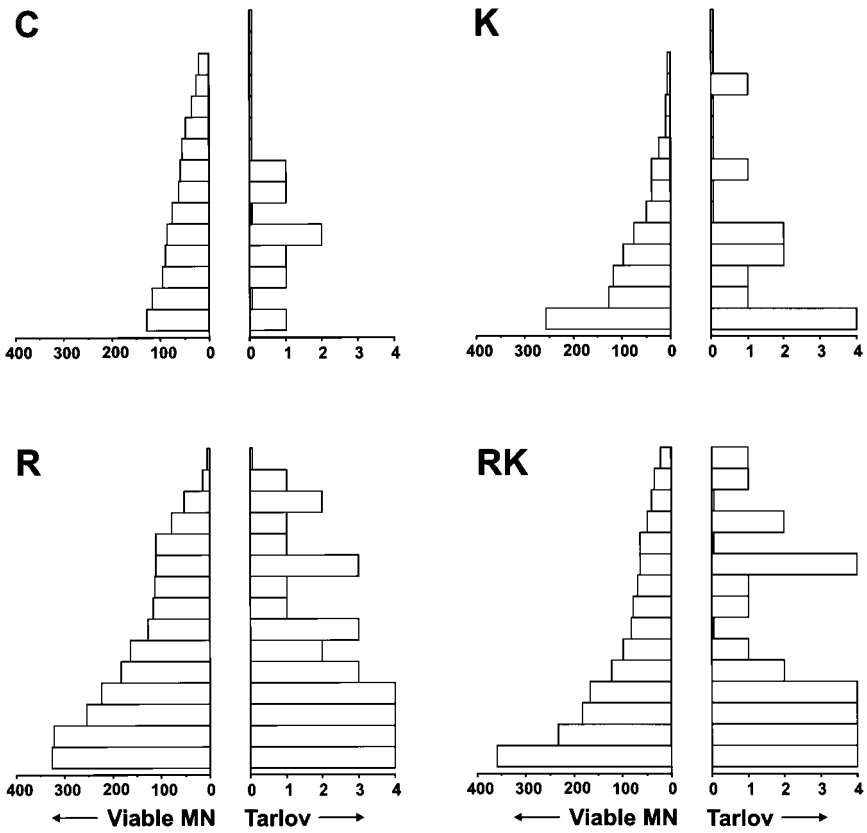


**Fig. 2. Histograms of neurologic (Tarlov) score and the total number of viable motoneurons in the ventral horn per animal in the four treatment groups. The number of motoneurons is shown at the left of each diagram, and neurologic score is shown at the right of each diagram. Neurologic score: 0, paraplegic with no lower-extremity function; 1, poor lower-extremity function, weak antigravity movement only; 2, some lower-extremity motor function with good antigravity strength but inability to draw legs under body or hop; 3, ability to draw leg under body and hop but not normally; 4, normal motor function. C = control; K = ketamine; R = riluzole; RK = riluzole plus ketamine; viable MN = total number of viable motoneurons in the ventral horn.**

In a recent study, a preischemic single 8-mg/kg dose of riluzole improved short-term (24-h) neurologic outcome and reduced the number of necrotic and TUNEL-stained neurons after SCI.[12] To allow for maturation of the infarcted spinal cord tissue, we assessed spinal cord injury 72 h after aortic occlusion. Our data suggest that, depending on the drug regimen, both deterioration and improvement of neurologic function can occur between 24 h and 72 h. As illustrated in table 3, some animals that were treated with saline in the postoperative period (groups C and K) showed worsening of neurologic function over time, whereas some riluzole-treated animals had better neurologic scores after 48 and 72 h compared with the early postoperative period (24 h). A possible explanation is that continuation of riluzole treatment into the postoperative period provided persistent inhibition of glutamate receptor activation.

*Riluzole and Ketamine*

In theory, inhibition of both the presynaptic and postsynaptic influences of glutamate-mediated excitotoxicity *via* the combined application of intraischemic riluzole and ketamine may result in an additional or synergistic neuroprotective effect. However, controversy remains regarding whether NMDA receptor blockade by ketamine can provide sufficient neuronal protection in focal cerebral ischemia and SCI.[1,21,22] The present data indicate that a 55-mg/kg dose of intraischemic ketamine in a temperature-controlled model for SCI does not improve neurologic outcome.

**Table 4. Infarction Volumes and Neuronal Cell Counts in the Treatment Groups**

| Group | Total Gray | Total Infarction | Infarction % | Viable Motoneurons | Eosinophilic Neurons |
|---|---|---|---|---|---|
| Control (n = 13) | 171.9 (158.1–205.3) | 84.9 (57.2–134.6) | 50.2 (31.0–69.2) | 62 (38–94) | 7 (1–34) |
| Riluzole (n = 15) | 203.3 (183.7–206.9) | 75.9 (7.9–112.1) | 41.7 (4.0–62.6) | 117* (86–213) | 6 (2–24) |
| Ketamine (n = 13) | 232.1 (209.9–241.1) | 122.4 (108.2–189.9) | 62.0 (48.6–77.5) | 39 (8–112) | 2 (0–5) |
| Riluzole–ketamine (n = 15) | 216.2 (203.2–242.5) | 110.2 (42.0–154.2) | 44.8 (18.7–70.0) | 78 (53–156) | 11 (3–23) |

Data are expressed as medians, with interquartile ranges in parentheses. Total gray = total volume of gray matter ($mm^3$); total infarction = total volume of infarcted gray matter ($mm^3$); infarction % = relative volume of infarcted gray matter.

* $P < 0.05$ as compared with controls.

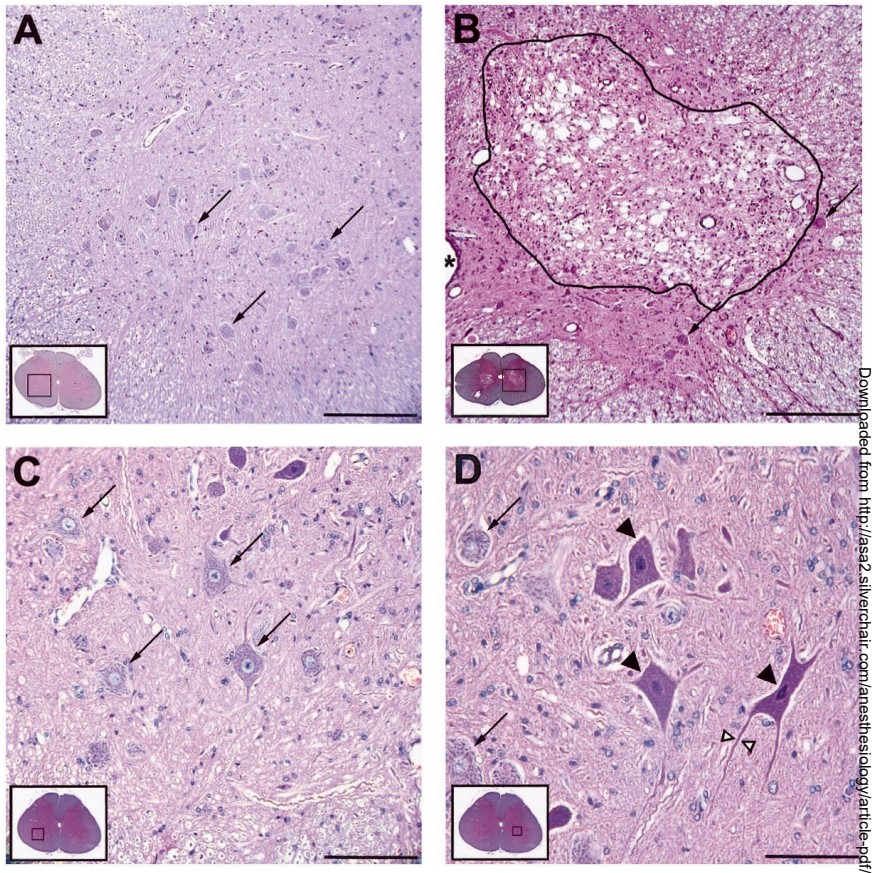


**Fig. 3. Representative photomicrographs of spinal cord segments at L3–L5. All animals had a normal motor function at 72 h after reperfusion. (*Inset*) Low-power overview. The bottom of each picture is the ventral side of the spinal cord. (*A*) Right ventral horn without infarctions of a riluzole-treated animal. Note numerous viable motoneurons (arrows). (*B*) Massive infarction of a large part of the intermediate and ventral gray matter of a riluzole-treated animal (same as indicated in fig. 1). Infarcted gray matter is delineated. Note the peripheral rim of surviving motoneurons (arrows). The central canal is indicated (*). (*C*) Viable motoneurons (arrows) display a clear nucleolus, Nissl substance (basophilic stippling of the cytoplasm), and large neurites (open arrowheads). (*D*) Several eosinophilic neurons are present in the ventral horn. Note the darkly stained cytoplasm of dead neurons (arrowheads) compared with the fine granular cytoplasm and Nissl substance of the viable cells (arrows). Eosinophilic neurites are clearly visible (open arrowheads). Note nuclear condensation and the absence of nucleoli in eosinophilic neurons. Micrographs were taken from hematoxylin and eosin–stained paraffin-embedded sections. Scale bars represent 265 μm (*A* and *B*), 134 μm (*C*), and 84 μm (*D*).**

One possible explanation is that ketamine, which has a very short half-life in the brain (50 min in rats),[23] may have been present for an insufficient period to provide protection in our model.

In contrast to riluzole, clinically relevant concentrations of ketamine were not present in the postoperative period; continued postoperative administration of ketamine was not feasible because of its anesthetic and hypotensive effects. Riluzole-treated animals showed better hind limb motor function than did controls during every neurologic evaluation (24, 48, and 72 h). However, no differences in neurologic scores were present between controls and ketamine-treated animals throughout the survival period. This makes it less probable that continuation of ketamine treatment in the postoperative phase, using the same administration regime as that with riluzole-treated animals, would have improved neurologic and histopathologic outcome.

Low ketamine concentrations in the postischemic period might explain the lack of additional protection with combined riluzole and ketamine. Another explanation might be that the suppression of glutamatergic neurotransmission with riluzole was so efficient that a second NMDA receptor blocker did not further reduce excitotoxic damage. However, because ketamine alone did not improve neurologic function in this study, the latter hypothesis seems unlikely.

As with cerebral ischemia, the neuronal damage following transient SCI is thought to involve glutamate excitotoxicity.[24,25] Riluzole may reduce glutamatergic

**Table 5. Infarction Volumes and Neuronal Cell Counts in Three Motor Function Groups**

| Group | Total Gray | Total Infarction | Infarction % | Viable Motoneurons | Eosinophilic Neurons |
|---|---|---|---|---|---|
| Paraplegia (n = 17) | 203.3 (166.5–239.1) | 126.2* (102.0–189.9) | 71.3* (50.1–78.2) | 39 (15–66) | 1† (0–19) |
| Paraparesis (n = 29) | 201.9 (183.2–233.6) | 110.2* (72.8–135.1) | 50.1* (40.5–64.7) | 89 (57–117) | 3‡ (2–12) |
| Normal (n = 10) | 206.9 (206.9–217.4) | 0 (0–9.8) | 0 (0–3.9) | 243 (184–323) | 24 (8–67) |

Data are expressed as medians, with interquartile ranges in parentheses. Volumes are shown in $mm^3$. Paraplegia: Tarlov = 0; paraparesis: Tarlov = 1–3; normal motor function: Tarlov = 4. Total gray = total volume of gray matter ($mm^3$); total infarction = total volume of infarcted gray matter ($mm^3$); infarction % = relative volume of infarcted gray matter.

* $P < 0.001$, † $P < 0.05$, ‡ $P < 0.01$ as compared with normal motor function.

neurotransmission,[26,27] possibly *via* a stabilizing effect on inactivated voltage-dependent sodium channels[28] located on glutamatergic nerve terminals[29] or by interaction with a GTP-binding signal transduction protein (G-protein).[30] In the caudate nucleus of the cat, riluzole reduced spontaneous release of glutamate,[7] but Kwon *et al.*[31] did not find an inhibition of extracellular hippocampal glutamate accumulation by riluzole in a rabbit model of global cerebral ischemia. Postsynaptic effects might also contribute, at least in part, to the neuroprotective mechanism, because rat motoneurons were protected from glutamate-induced degeneration *in vitro* by application of riluzole.[32] Furthermore, riluzole appears to have a direct inhibitory effect on the NMDA receptor.[8] Taken together, these results suggest that neuroprotection by riluzole involves both decreased glutamate release and inhibition of its deleterious effects.

*Temperature*

The current data show that spinal cord protection by periischemic administration of riluzole is not caused by drug-induced reduction of core temperature. A temperature decrease of 2°C can significantly reduce neuronal necrosis in the ischemic brain,[33] possibly by reducing the intraischemic release of excitatory amino acids.[34] Mild hypothermia may result from sedative side-effects of some neuroprotectants (*e.g.*, NMDA antagonists).[35] This implies that accurate maintenance of temperature at the site of the ischemic insult is necessary to rule out possible influences on temperature regulation in experimental studies of neuroprotective agents during ischemia.[36,37] In the current study, paraspinal muscle temperature was maintained at 38°C throughout the operative procedure. The intraperitoneal infusion of riluzole did not reduce rectal temperature 1 h after administration of the substance.

*Histopathology*

In general, severity of injury as assessed using Tarlov scores correlated well with the extent of gray matter infarctions and was inversely related to the number of viable motoneurons. In animals that had significant impairment of motor function, there was no difference in gray matter necrosis among treatment groups. However, riluzole increased the proportion of animals that had normal motor function, and in these animals, necrosis was absent and more viable motoneurons were present. Quantification of viable motoneurons in the ventral horn may correlate better with the clinical evaluation of motor function than measurements of necrotic damage of the spinal cord gray matter. Matsumoto *et al.*[38] described the preservation of motoneurons in the anterior spinal cord of rabbits that underwent transient SCI, which correlated well with the neurologic outcome. The current results are consistent with *in vitro* studies that provide evidence for the selective protection of motoneurons by riluzole.[32] Animals that had normal motor function (irrespective of the treatment received) showed more eosinophilic neurons than did paraparetic or paraplegic animals. One possible explanation is the more difficult identification of eosinophilic neurons in necrotic areas because of the disappearance of well-defined cellular morphology.

These data indicate that the effects of riluzole on spinal cord histopathology are predominantly an increased proportion of undamaged animals—with corresponding lack of motor function deficits—rather than a global reduction of infarction volume.

*Potential Use in Humans*

Surgical settings with a high probability of transient neuronal ischemia, for example, patients undergoing surgery for intracranial aneurysms or temporary cross-clamping of the aorta, might benefit from adjuncts (*e.g.* hypothermia, pharmacologic agents) that can increase ischemic tolerance of the neuronal populations at risk. However, clinical application of several agents that were protective in experimental neuronal ischemia is hampered by their side-effects.[39]

Oral riluzole is well tolerated in humans.[9] If the neuroprotective effects of riluzole can be confirmed in other models and species, a clinical trial of spinal cord protection, *e.g.*, during thoracoabdominal aneurysm surgery might be considered.

## Conclusions

The results of the current study indicate that treatment with riluzole before and after transient SCI can improve neurologic outcome. In contrast, periischemic ketamine did not provide neuroprotection, and combined treatment with riluzole and ketamine was no more effective than riluzole alone.

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