Delayed Paraplegia After Spinal Cord Ischemic Injury Requires Caspase-3 Activation in Mice

Manabu Kakinohana, MD, PhD; Kotaro Kida, MD, PhD; Shizuka Minamishima, MD; Dmitriy N. Atochin, MD, PhD; Paul L. Huang, MD, PhD; Masao Kaneki, MD, PhD; Fumito Ichinose, MD, PhD

Background and Purpose—Delayed paraplegia remains a devastating complication after ischemic spinal cord injury associated with aortic surgery and trauma. Although apoptosis has been implicated in the pathogenesis of delayed neurodegeneration, mechanisms responsible for the delayed paraplegia remain incompletely understood. The aim of this study was to elucidate the role of apoptosis in delayed motor neuron degeneration after spinal cord ischemia.

Methods—Mice were subjected to spinal cord ischemia induced by occlusion of the aortic arch and left subclavian artery for 5 or 9 minutes. Motor function in the hind limb was evaluated up to 72 hours after spinal cord ischemia. Histological studies were performed to detect caspase-3 activation, glial activation, and motor neuron survival in the serial spinal cord sections. To investigate the impact of caspase-3 activation on spinal cord ischemia, outcome of the spinal cord ischemia was examined in mice deficient for caspase-3.

Results—In wild-type mice, 9 minutes of spinal cord ischemia caused immediate paraplegia, whereas 5 minutes of ischemia caused delayed paraplegia. Delayed paraplegia after 5 minutes of spinal cord ischemia was associated with histological evidence of caspase-3 activation, reactive astrogliosis, microglial activation, and motor neuron loss starting at approximately 24 to 48 hours after spinal cord ischemia. Caspase-3 deficiency prevented delayed paraplegia and motor neuron loss after 5 minutes of spinal cord ischemia, but not immediate paraplegia after 9 minutes of ischemia.

Conclusions—The present results suggest that caspase-3 activation is required for delayed paraplegia and motor neuron degeneration after spinal cord ischemia. (Stroke. 2011;42:2302-2307.)

Key Words: apoptosis ■ cleaved caspase-3 ■ delayed neuronal death ■ delayed paraplegia ■ spinal cord ischemia

Delayed paraplegia is a devastating complication of spinal cord ischemia (SCI), which can occur after thoracic and abdominal aortic surgery for a variety of aortic pathologies, including aneurysm and trauma. Rates of immediate and delayed-onset neurological deficits after major thoracic aortic repairs range between 4% and 11%. Of all incidences of paraplegia (immediate and delayed combined) associated with aortic surgery, the reported incidence of delayed paraplegia varied from 12% to 73%. Although introduction of several adjunct procedures, including cerebrospinal fluid drainage, reduced the incidence of delayed paraplegia after aortic surgery, the incidence of delayed paraplegia has not changed.

Although immediate paraplegia is thought to be caused by an irreversible ischemic neuronal injury in the spinal cord, the mechanism responsible for delayed paraplegia is incompletely understood. Several potential mechanisms responsible for the development of delayed paraplegia have been proposed, including delayed apoptotic neuronal death executed by caspase-3 activation. Nonetheless, the role of motor neuron apoptosis and caspase-3 activation in the pathogenesis of delayed paraplegia remains controversial; some studies show the presence of apoptosis in the spinal cord of animals exhibiting delayed paraplegia, whereas others do not. Because the majority of these studies examined the role of apoptosis using immunohistochemical detection of caspase-3 and/or DNA fragmentation, no causal relationship between caspase-3 activation and delayed motor neuron death has been established to date. Furthermore, elucidation of the role of apoptosis in delayed paraplegia has been hindered by the lack of reproducible animal models in which genetic modification can be exploited to determine the molecular mechanisms.

To define the molecular mechanisms responsible for the delayed paraplegia after SCI, we have recently developed a...
mouse model of delayed paraplegia by modifying a previously reported mouse model of SCI. This model is unique in which mice that are subjected to SCI initially recover from surgery and anesthesia and exhibit ability to walk for approximately 24 hours. Subsequently, however, all mice develop delayed paraplegia starting at approximately 30 to 36 hours after surgery. Using this robust model, we sought to determine the role of apoptotic neuronal death in immediate and delayed paraplegia after SCI. We report that caspase-3 activation is required for delayed paraplegia but not for immediate paraplegia in mice.

Materials and Methods

Mouse Model of SCI

After approval by the Massachusetts General Hospital Subcommittee on Research Animal Care, male wild-type mice (WT, C57BL/6J, 8 to 10 weeks old; Jackson Laboratory, Bar Harbor, ME) and male mice deficient for caspase-3 backcrossed onto C57BL/6 background >10 generations (caspase-3<sup>-/-</sup>, 8 to 10 weeks old)<sup>9</sup> were anesthetized with isoflurane and subjected to SCI according to the method described by Lang-Lazdunski and colleagues with modifications.<sup>9</sup> See http://stroke.ahajournals.org for the details of surgical procedures, measurements of physiological parameters, quantal bioassay, and histological studies.

Assessment of Motor Neuron Function

Motor function was quantified serially at pre-SCI, 8, 24, 48, and 72 hours after SCI by the Basso Mouse Scale (BMS).<sup>11</sup> The maximum deficit is indicated by a score of 0. Although BMS score < 6 (0 to 5) indicates paraplegia, BMS score ≥ 6 (6 to 9) indicates ability to walk.

Statistics

Parametric data were presented as mean±SD. Analysis of variance followed by Bonferroni and Tukey-Kramer tests or Student t test was used to compare parametric data. The quantal bioassay was based on logistic analysis.<sup>12</sup> The difference between the probability of producing delayed and immediate paraplegia in 50% of mice (P50d and P50i, respectively) was graphically demonstrated by computer construction of an ischemic duration probability of paraplegia curve for each group.<sup>13</sup> Changes in BMS were analyzed by 2-way repeated-measures analysis of variance followed by Tukey test. The number of viable neurons between experimental groups at individual time points was compared with Mann-Whitney U test. Probability values <0.05 were considered significant.

Results

SCI Causes Immediate or Delayed Paraplegia

All mice subjected to 9 minutes SCI showed immediate paraplegia that persisted up to 72 hours after SCI (P<0.01 versus sham and pre-SCI; Figure 1). All mice subjected to 5 minutes SCI showed modest and transient motor weakness (BMS 6 to 9) at 30 minutes after reperfusion followed by gradual recovery over the next 24 to 30 hours (Figure 1). Up to approximately 8 hours after 5 minutes SCI, motor deficit manifested as ataxia and partial weakness in place-stepping reflex with preserved ability to walk (P<0.05 versus 9 minutes SCI at 8 and 24 hours after SCI; Figure 1). The motor function of lower extremities of mice subjected to 5 minutes SCI gradually worsened starting at approximately 30 hours after reperfusion and all mice exhibited complete paraplegia by 48 hours after reperfusion (Figure 1). Long-term outcomes after SCI (6 weeks) were examined in subgroups of mice that were subjected to 9 or 5 minutes SCI. Although all mice that were subjected to 9 minutes SCI died within 7 days, 20% of mice survived up to 6 weeks after 5 minutes of SCI (Supplemental Figure I). Once they became paraplegic, no mice showed any recovery of hind limb motor function until death or up to 6 weeks after SCI of either duration. Sham-
operated mice did not show any neurological deficits throughout the experiments (Figure 1).

Quantal Bioassay for the Relationship Between the Duration of SCI and Neurological Function

The incidence of paraplegia (BMS=0 to 5) increased as the duration of SCI increased (Supplemental Table I; Figure 2). In the current model, <3 minutes of SCI appeared to be well tolerated that did not cause motor deficit. Although many mice exhibited ability to walk (BMS=6 to 9) up to 24 hours after SCI after shorter durations of aortic clamping (from 3.5 to 7.5 minutes), the majority of the mice developed complete paraplegia by 48 hours of reperfusion (delayed paraplegia). All mice subjected to >8 minutes of SCI showed motor deficit in the hind limb immediately after recovering from anesthesia without any recovery up to 72 hours after SCI (immediate paraplegia). Quantal bioassay analysis revealed that the P50i and P50d were 6.6±0.1 minutes and 3.5±0.1 minutes, respectively (Figure 2, P<0.01).

Time Course of Neurodegeneration After SCI

In the spinal cord of mice subjected to 9 minutes SCI, neurons in the ventral horn started to degenerate at 8 hours after SCI. Extensive neuronal loss appeared to have completed by 24 hours of reperfusion without further changes up to 72 hours after 9 minutes SCI (Figure 3A–B). In contrast, 5 minutes SCI did not affect the number and appearance of neurons in the ventral horn at 8 and 24 hours after reperfusion (Figure 3A–B). However, the ventral horns of mice subjected to 5 minutes SCI exhibited extensive cellular loss with marked cavitation at 48 and 72 hours after SCI. Although the structure of the ventral horn appeared to be better preserved at 72 hours after 9 minutes of SCI than after 5 minutes of SCI, extensive neuronal loss after SCI of both durations was confirmed by the absence of NeuN-positive neurons at 72 hours after 9 or 5 minutes of SCI (Figure 3C).

SCI Activates Astrocytes and Microglia

To examine the mechanisms responsible for the delayed extensive cavitation observed in the spinal cord of mice...
subjected to 5 minutes SCI, activation of astrocytes and microglia was assessed by immunohistochemical staining of glial fibrillary acidic protein and ionized calcium binding adaptor molecule 1, respectively. Although only few reactive astrocytes and activated microglia were observed at 8 and 24 hours after 5 minutes SCI (Figure 4A), the number of reactive astrocytes and activated microglia markedly increased at 48 hours after 5 minutes SCI (Figure 4A). In contrast, in the spinal cord of mice subjected to 9 minutes SCI, no reactive astrocyte or activated microglia were observed up to 48 hours after reperfusion (Figure 4A). These results suggest that delayed inflammatory reaction associated with glial activation may have contributed to the extensive spinal cord damage and delayed paraplegia after 5 minutes SCI.

**Caspase-3 Activation Precedes the Onset of Delayed Paraplegia**

A small number of cleaved caspase-3-positive neurons was observed in the ventral horn of the spinal cord of mice at 8 hours after 9 minutes SCI (Figure 4B). However, only a few cleaved caspase-3-positive neurons were found at 24 and 48 hours after 9 minutes SCI, presumably because of the extensive necrosis and disappearance of the spinal ventral neurons. In contrast, the number of ventral horn neurons with positive cleaved caspase-3 immunoreactivity markedly increased at 24 and 48 hours after 5 minutes SCI (Figure 4). These results suggest caspase-3 activation precedes the onset of extensive neuroinflammation, neurodegeneration, and delayed paraplegia after 5 minutes SCI.

**Caspase-3 Deficiency Prevents Delayed Paraplegia in Mice**

To determine the role of caspase-3 on the immediate and delayed onset paraplegia after SCI, we examined outcomes of SCI in caspase-3−/− mice. There was no difference in physiological parameters, including regional spinal cord blood flow during SCI between WT and caspase-3−/− mice (Supplemental Tables II, III, and IV). Similar to WT mice, caspase-3−/− mice that were subjected to 9 minutes SCI exhibit flaccid paraplegia immediately after recovery from anesthesia (Figure 5). Histopathologic analysis revealed extensive neuronal loss in the spinal cord of caspase-3−/− mice harvested at 72 hours after 9 minutes SCI (data not shown). In contrast to WT mice that developed delayed paraplegia after 5 minutes SCI, caspase-3−/− mice that were subjected to 5 minutes SCI exhibited near normal motor function of lower extremity up to 72 hours after reperfusion (P<0.01 versus WT 48 and 72 hours after 5 minutes SCI; Figure 5). Histopathologic analysis of the spinal cord of caspase-3−/− mice subjected to 5 minutes SCI revealed normal appearances of the spinal ventral gray matter at 72 hours after reperfusion (Figure 6A). There was a statistically significant difference (P<0.01) in the number of Nissl-positive spinal cord neurons at 72 hours after reperfusion between WT and caspase-3−/− mice subjected to 5 minutes SCI (Figure 6B).
spinal ventral neurons at 72 hours after 5 minutes SCI in WT baboon,15 pig,16 and rat17 have been used to study SCI. The injury.

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caspase-3 activa-

and microglial activation as well as marked cavitation in the

ventral horn at 48 hours after reperfusion. Caspase-3 activa-

tion supports this hypothesis. In contrast to 5 minutes SCI,

no cavitation or glial activation was observed after 9 minutes

SCI. Although the reason for the differing impact of 5 and 9

minutes SCI may be multifactorial, it is conceivable that 9

minutes of ischemic insult is too severe for any cells to

survive in the ischemic spinal cord regions.

Delayed paraplegia and neurodegeneration after 5 minutes

SCI were associated with marked cavitation in the ventral

horn at 48 and 72 hours after reperfusion. The extensive

cellular loss was associated with reactive astrogliosis and

microglial activation at 48 hours after 5 minutes SCI. Al-

though the role of activated astrocytes and microglia after

SCI is incompletely understood, it is possible that necrosis

and/or apoptosis activates proinflammatory and phagocytotic

activity of glial cells promoting tissue damage.22 The current

observation that caspase-3 activation preceded the glial acti-

vation supports this hypothesis. In contrast to 5 minutes SCI,

cavitation or glial activation was observed after 9 minutes

SCI. Although the reason for the differing impact of 5 and 9

minutes SCI may be multifactorial, it is conceivable that 9

minutes of ischemic insult is too severe for any cells to

survive in the ischemic spinal cord regions.

The role of caspase-3 activation and apoptosis in the

pathogenesis of delayed motor neuron death has been impli-
cated primarily based on immunohistochemical detection of

cleaved caspase-3 in the spinal cord of rabbits that exhibited

delayed paraplegia after SCI.4–6 However, the role of

caspase-3 in delayed motor degeneration has been questioned

because of the failure of a conventional caspase inhibitor to

prevent delayed paraplegia7 and lack of caspase-3 activation

and apoptosis in the spinal cord of rabbits.8 These conflicting

results are at least in part due to the limited potency and/or
toxicity of chemical caspase inhibitors and lack of reproduc-
ible and standardizable animal models of SCI. In the current

study, using caspase-3$^{-/-}$ mice, we demonstrated that

caspase-3 is required for the SCI-induced development of
delayed paraplegia, but not immediate paraplegia, in mice.

Time-dependent changes of the SCI-induced caspase-3 activ-
tion in the spinal cord support the critical role of caspase-3 in

the pathogenesis of delayed paraplegia (see Figure 4). Al-

though the precise mechanism whereby caspase-3 defi-

ciency prevents SCI-induced delayed paraplegia remains to

be determined, our results suggest that inhibition of apoptotic

cell death and glial activation in caspase-3$^{-/-}$ mice contrib-
uted to the preserved spinal cord neurons after 5 minutes of

Discussion

In the present study, we found that 9 and 5 minutes of SCI
produced immediate- and delayed-onset paraplegia, respec-
tively, in mice. Once they became paraplegic, no mice
showed any recovery of motor function in this model up to 6
weeks after SCI of either duration. Although degeneration of
the spinal cord neurons and motor deficit became apparent
8 hours after 9 minutes SCI, neurodegeneration and paralysis
did not manifest until 36 to 48 hours after 5 minutes SCI in
WT mice. The delayed neurodegeneration and paraplegia
after 5 minutes SCI were associated with reactive astrogliosis
and microglial activation as well as marked cavitation in the
ventral horn at 48 hours after reperfusion. Caspase-3 activa-
tion after 5 minutes SCI precedes the onset of glial activation
and extensive neuronal loss. Of note, although 9 minutes SCI
produced immediate paraplegia in caspase-3$^{-/-}$ mice,
caspase-3 deficiency prevented delayed motor neuron loss and
paraplegia after 5 minutes SCI. These observations suggest that

caspase-3 activation is required for delayed

neurodegeneration and paraplegia after spinal cord ischemic

injury.

A variety of animal models including the dog,14 rabbit,4
baboon,15 pig,16 and rat17 have been used to study SCI. The
rabbit model of SCI has been particularly popular because it
can produce delayed and immediate paraplegia by tempo-
anarily occluding the infrarenal aorta. However, the arterial blood

supply of the spinal cord in rabbit is almost purely segmental
and different from that in humans.18,19 In rats and mice,9 1
anterior and 2 posterior spinal arteries supply the spinal cord,
which is similar to humans. Moreover, availability of the
variety of genetically altered mouse strains is a clear advan-
tage of mouse models of human diseases compared with rats
and rabbit.
SCI. To elucidate the role of caspases in the pathogenesis of delayed paraplegia, further studies using genetically altered mouse models and new-generation caspase inhibitors are warranted.

**Conclusions**

The current study demonstrates that SCI in mice can cause immediate or delayed-onset motor neuron degeneration and paraplegia depending on the duration of ischemia. Our results also provide definitive evidence that caspase-3 activation is required for the development of delayed paraplegia after 5 minutes of SCI in mice. These observations have important clinical implications suggesting the preventive effects of caspase-3 inhibition in SCI induced by surgery or trauma. Furthermore, the unique mouse model of delayed paraplegia described here provides an important experimental platform to further examine the molecular mechanisms of delayed motor neuron degeneration.

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**Disclosures**

None.

**References**


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Supplemental Methods

Mouse model of spinal cord ischemia

The operative procedures to produce transient SCI in mice were performed according to a method described by Lang-Lazdunski and colleagues\(^1\) with some modifications. After the animals were weighed, anesthesia was induced by 5% isoflurane, and trachea was intubated by 20 G custom made catheter for mechanical ventilation. Mice were positioned in the spine position and anesthesia was maintained by 1.5% to 2% isoflurane with 100% oxygen. Paravertebral muscle temperature was measured by a thermocouple placed at the level of L1-L3 and maintained at 37.5 ± 0.2°C during surgery using a heating pad. Subsequently, PE-10 catheter was inserted into the left femoral artery to monitor distal arterial pressure. A ventral midline cervicothoracic incision was made, submaxillary glands were retracted, and the chest wall was incised from the apex of the manubrium caudal along the left sternal border, to the second rib. The thymus was retracted superiorly, and the aortic arch was gently isolated between the brachiocephalic artery and the left subclavian artery (LSA), avoiding the vagus nerve and the left recurrent laryngeal nerve. Heparin (1000 IU/kg) was injected via the left femoral arterial catheter. Then, under direct vision, the first clip was placed on the aortic arch between the left common carotid artery and the LSA, and then the second clip was placed on the origin of the LSA (within 30 seconds). The completeness of the occlusion was ascertained by an immediate and sustained loss of any detectable pulse pressure in the femoral artery pressure tracing. After ischemia, the clips were removed, and the chest was closed in layers. Protamine sulfate (1 mg) was then administered subcutaneously. At 10 minutes of reperfusion, the arterial catheter was removed, incisions were closed, and animals were allowed to recover from anesthesia. In sham-operated mice, all surgical procedures were performed as described, but no clips were placed. All mice were placed in a cage kept at 30 - 31°C for the following 2 hours.

These modifications enabled us to enhance the completeness of the surgical isolation and clamping of aortic arch that resulted in a highly reproducible reduction of rSCBF down to less than 10% of baseline compared to ~30% in the Lang-Lazdunski study.\(^1\) While 11 min of aortic occlusion resulted in immediate paraplegia in ~80% of
mice in the Lang-Lazdunski study, 1 9 min of SCI was sufficient to produce immediate paraplegia in 100 % of mice at 72h after reperfusion in the current study with minimum operative mortality.

**Measurements of physiological parameters**

In addition to paravertebral muscle temperature, rectal temperature was also monitored and recorded periodically (before ischemia, at the end of ischemia, and 10 min of reperfusion) as core temperature during surgery. Blood gas analysis was performed before aortic occlusion and at 10 min of reperfusion. Arterial blood gases and pH were measured before ischemia and 10 minutes of reperfusion in 50µL samples obtained from the left femoral arterial catheter by a blood gas /pH analyzer (IRMAs TRUPOINT™, ITC. USA).

We used a qualitative real-time measure of regional spinal cord blood flow (rSCBF) by laser-Doppler flowmetry (PF2B, Perimed) with a 0.8-mm fiberoptic extension. As described by Lang-Lazdunski et al., 1 the probe was affixed perpendicularly as much as possible on the intervertebral ligament surface between vertebra L1 and L2 through a limited skin incision.

**Quantal bioassay for the relationship between the duration of SCI and neurological function**

For the quantal bioassay of the relationship between the duration of SCI and neurologic function2,3, the duration of SCI was selected to span all grades of neurologic function ranging from walking (BMS = 6 - 9) to paraplegia or paraparesis (BMS = 0 - 5). Based on our pilot experiments, the duration of SCI for individual animals was varied from 1 min up to 10 min. The P50i and P50d represent the duration of ischemia (in minutes) associated with 50% probability of immediate and delayed paraplegia, respectively. The onset of neurologic deficit (BMS = 0 - 5) was considered immediate if it was present at the initial examination (2 hrs after reperfusion) and delayed if the deficit occurred after a period during which mice exhibited the ability to walk (BMS = 6 - 9).
Histological studies

After SCI, lumbar enlargement of the spinal cord was perfusion fixed and embedded in paraffin. Spinal cord embedded in paraffin was sectioned to 5 μm thickness and 50 sections of each spinal cord were divided on ten slide glasses (five sections each on one slide glass). For each histopathological analysis described below, one slide glass of each spinal cord was chosen randomly by the investigator (KK) without the knowledge of the identity of the sample.

Detection and quantification of viable neurons with Nissl staining—Viability of neurons in paraffin-embedded spinal cord sections obtained from mice subjected to sham operation or 9 or 5 min of SCI were evaluated with Nissl staining. Cells that contained Nissl substance in the cytoplasm, loose chromatin, and prominent nucleoli were considered to be viable. For quantitative analysis, the number of Nissle-positive neuron in the spinal ventral horn was counted in a randomly-chosen section under high-power magnification (200x) by an investigator (KK) blinded as to the identity of mice. N=4 for Sham, 8, 24, and 48h after 9 or 5 min SCI. N=6 for 72h after 5 min SCI. N=7 for 72h after 9 min SCI.

Immunohistochemical detection of glial activation and viable neurons—Sections were incubated for 1h in blocking solution and incubated overnight at 4°C with primary antibodies against markers of activated astrocytes (glial fibrillary acidic protein, GFAP, 1:500, Dako, Carpinteria, CA) or activated microglia (ionized calcium binding adaptor molecule 1, Iba-1, 1:500, Wako Chemicals USA, Richmond, VA). After rinsing, sections were incubated with secondary antibodies for 1h: Rhodamine RedTX-conjugated goat anti-rabbit antibody (for GFAP, 1:200, Jackson ImmunoResearch, West Grove, PA) or Alexa Fluor 488 goat anti-rabbit antibody (for Iba-1, 1:200, Invitrogen, Carlsbad, CA). Viable neurons were detected by mouse anti-neuronal nuclei (NeuN) conjugated to Alexa Fluor 488 (1:100, Millipore, Billerica, MA). The fluorescence images were captured using appropriate filters with a fluorescence microscope (Nikon ECLIPSE TE-2000-S).

Detection of cleaved caspase 3—Activation of caspase-3 was assessed by immunohistochemistry in paraffin-embedded spinal cord sections using a rabbit polyclonal antibody against cleaved caspase-3 (1:50, Cell Signaling) according to the protocol recommended by the manufacturer.
### Supplemental Tables

**Table S1. Comparison of the Effects of Spinal Cord Ischemic Time on Neurological Outcome**

<table>
<thead>
<tr>
<th>Duration of SCI (min)</th>
<th>Total number of mice</th>
<th>Number of mice exhibiting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Normal motor function</td>
</tr>
<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
<td>4</td>
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<td>9</td>
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<td>10</td>
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</table>

**Table S2. Physiological variables –Immediate paraplegia–**

<table>
<thead>
<tr>
<th>Ischemic Time (min)</th>
<th>Wild-type</th>
<th>Caspase-3−/−</th>
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</thead>
<tbody>
<tr>
<td>Femoral artery pressure (mmHg)</td>
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<td></td>
</tr>
<tr>
<td>Pre-ischemia</td>
<td>79±5</td>
<td>82±6</td>
</tr>
<tr>
<td>Intra-ischemia</td>
<td>8±1</td>
<td>8±1</td>
</tr>
<tr>
<td>10 min of reperfusion</td>
<td>75±19</td>
<td>87±26</td>
</tr>
<tr>
<td>Paravertebral muscle temperature (ºC)</td>
<td></td>
<td></td>
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<tr>
<td>Pre-ischemia</td>
<td>37.4±0.1</td>
<td>37.4±0.1</td>
</tr>
<tr>
<td>Intra-ischemia</td>
<td>37.4±0.1</td>
<td>37.4±0.1</td>
</tr>
<tr>
<td>10 min of reperfusion</td>
<td>37.4±0.1</td>
<td>37.5±0.2</td>
</tr>
<tr>
<td>Rectal temperature (ºC)</td>
<td></td>
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<tr>
<td>Pre-ischemia</td>
<td>36.6±0.3</td>
<td>36.9±0.3</td>
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<tr>
<td>Intra-ischemia</td>
<td>36.8±0.4</td>
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<tr>
<td>10 min of reperfusion</td>
<td>36.9±0.2</td>
<td>36.8±0.2</td>
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</table>
Table S3. Physiological variables –Delayed paraplegia-

<table>
<thead>
<tr>
<th></th>
<th>Wild-type</th>
<th>Caspase-3&lt;sup&gt;-/-&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic Time (min)</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Femoral artery pressure (mmHg)</td>
<td></td>
<td></td>
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<tr>
<td>Pre-ischemia</td>
<td>83±7</td>
<td>79±6</td>
</tr>
<tr>
<td>Intra-ischemia</td>
<td>7±1</td>
<td>5±1</td>
</tr>
<tr>
<td>10 min of reperfusion</td>
<td>84±9</td>
<td>87±9</td>
</tr>
<tr>
<td>Paravertebral muscle temperature (ºC)</td>
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<tr>
<td>Pre-ischemia</td>
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<td>37.6±0.1</td>
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<tr>
<td>Intra-ischemia</td>
<td>37.4±0.1</td>
<td>37.5±0.0</td>
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<tr>
<td>10 min of reperfusion</td>
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<td>37.3±0.1</td>
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<tr>
<td>Rectal temperature (ºC)</td>
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<tr>
<td>Pre-ischemia</td>
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<td>37.1±0.4</td>
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<td>Intra-ischemia</td>
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<td>37.5±0.3</td>
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<tr>
<td>10 min of reperfusion</td>
<td>36.9±0.3</td>
<td>36.9±0.2</td>
</tr>
</tbody>
</table>

Table S4. Regional spinal cord blood flow in Wild-type and Caspase-3<sup>-/-</sup> mice

<table>
<thead>
<tr>
<th></th>
<th>Wild-type</th>
<th>Caspase-3&lt;sup&gt;-/-&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>rSCBP (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ischemia</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Intra-ischemia</td>
<td>8±4</td>
<td>7±3</td>
</tr>
<tr>
<td>10 min of reperfusion</td>
<td>91±14</td>
<td>101±13</td>
</tr>
</tbody>
</table>

rSCBF : regional spinal cord blood flow
Figure S1. Long-term survival rates of mice subjected to 5 or 9 min of spinal cord ischemia (SCI). N=10 in each group.
Supplemental Reference List


