Spinal Cord Ischemia
Development of a Model in the Mouse

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Background and Purpose—Spinal cord ischemia with resulting paraplegia is a devastating complication of thoracoabdominal aortic surgery. Experimental models of spinal cord ischemia have been developed in primate, dog, pig, rabbit, and rat with variable reproducibility, but none has been developed in mouse. Because genetically engineered mice have become important to examine the impact of specific genes in ischemic pathophysiology, we sought to develop a reproducible mouse model of spinal cord ischemia.

Methods—C57BL/6NCrlBR mice were subjected to cross-clamping of the aortic arch, left subclavian artery, and internal mammary artery for 9 minutes (group A; n = 8) or 11 minutes (group B; n = 29) followed by reperfusion for 24 or 48 hours. Mean distal arterial blood pressure (left femoral artery) and lumbar (L1) spinal cord blood flow (laser-Doppler flowmetry) were measured for the duration of the procedure. The arterial blood supply of the spinal cord was visualized by intravascular perfusion of carbon black ink. We evaluated motor function in the hind limbs at 0, 1, 3, 6, and 24 hours after reperfusion using a rating scale of 0 (normal function) to 6 (total absence of movement). Spinal cord histopathology was evaluated after 24 and 48 hours of reperfusion by Luxol fast blue–hematoxylin and eosin.

Results—The vascular anatomy of the mouse and human spinal cord appeared similar in that blood was supplied by 1 anterior and 2 posterior spinal arteries and heterosegmental radicular arteries. During combined occlusion of aortic arch and left subclavian artery, mean distal arterial blood pressure dropped to 10 ± 5 mm Hg, and spinal cord blood flow at the L1 level decreased to 27 ± 7% of baseline. All animals recovered from anesthesia with acute paraplegia. Animals in the 9-minute group (group A) showed steady recovery of hind limb function over the ensuing 24 hours, whereas the majority (80%) in the 11-minute group (group B) remained paralyzed with maximum deficit throughout the postoperative period. Mortality was 0% and 21% in groups A and B, respectively. Maximal ischemic damage was observed at the lower thoracic and higher lumbar spinal levels in both groups. In group A (9 minutes), tissue damage was mild, affecting predominantly dorsal horns and intermediate gray matter, whereas ventral horns were minimally involved. All mice in group B (11 minutes) showed extensive gray matter lesions particularly involving dorsal horns and intermediate areas; in ventral horns, >50% of motor neurons died. White matter lesions were present in the most severely damaged cords only.

Conclusions—Spinal cord ischemia caused by aortic arch plus left subclavian artery cross-clamping provides a mouse model useful for the study of spinal cord injury and of potential relevance to the complications following thoracoabdominal aortic surgery in humans. (Stroke. 2000;31:208-213.)

Key Words: ischemia ■ mice ■ spinal cord

Despite considerable improvements in surgical technique, paraplegia/paraparesis due to spinal cord ischemia remains a significant perioperative complication of thoracoabdominal aortic surgery.1-10 Animal models of spinal cord ischemia have been described in the literature with variable reproducibility.11-21 Zivin et al15,16 developed a highly reproducible model in the rabbit that has become the standard for the study of experimental spinal cord ischemia. In the rabbit, transient occlusion of the infrarenal segment of aorta causes paraplegia with a low rate of complications such as bowel or kidney ischemia and cardiac dilatation.15,16 However, the model differs somewhat from humans in that infrarenal aortic cross-clamping rarely causes paraplegia in humans because the thoracolumbar spinal cord is supplied by the Adamkiewicz artery, which arises in most cases above the renal arteries.22

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Genetically engineered mice are now used widely to study the impact of specific genes in neurological disease, including cerebral ischemia. However, little is known about spinal cord ischemia in the mouse. Therefore, we sought to develop a model of spinal cord ischemia in mice in which pathophysiology and treatment could be explored.

Materials and Methods

Animals
Male C57BL/6NCrBR (C57BL/6; weight, 21 to 26 g) mice were obtained from Charles River Laboratories (Wilmington, Mass) and allowed free access to laboratory chow and tap water in day/night-regulated quarters. Animal care and experiments complied with the “Principles of Laboratory Animal Care” (Guide for the Care and Use of Laboratory Animals, National Institutes of Health publication 86-23, 1985).

Anesthesia and Surgical Technique
After the animals were weighed, anesthesia was induced by 2.5% halothane. Anesthesia was maintained by inhalation of 1% to 1.5% halothane through a face mask driven by 100% O2 flow with the use of a Fluotec 3 vaporizer (Colonial Medical). Heparin (400 IU/kg) was injected subcutaneously. With the animals in the supine position, 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. Care was taken to avoid incising the left superior vena cava. A clip was first placed on the left internal mammary artery, which is known in humans to be an important collateral vessel to the spinal cord. The thymus was retracted superiorly, and the aortic arch was gently isolated between the left common carotid artery and the left subclavian artery (LSA), avoiding the vagus nerve and the left recurrent laryngeal nerve. Then, under direct vision, 1 clip was placed on the aortic arch between the left common carotid artery and the LSA, and then another clip was placed on the origin of the LSA (within 15 seconds) (Figure 1). The vascular occlusion was verified and maintained for 9 (n=8; group A) or 11 minutes (n=29; group B). Then the clips were removed, and the chest was closed in layers. After 15 minutes, mice were placed in a cage kept at 31°C for the following 3 hours. In preliminary experiments, we were unable to cause significant motor or sensory deficit after 12 minutes of clamping at the aortic isthmus.

Serial assessments were made of motor function in the hind limbs in all animals at 0, 1, 3, 6, and 24 hours. Credé’s maneuver was used for evacuation of the urinary bladder at least twice daily in paraplegic animals. Mice were killed at 24 hours (group A, n=7; group B, n=14) or 48 hours (group A, n=1; group B, n=6).

Physiological Parameters
Core temperature was continuously monitored with a flexible probe inserted into the rectum and maintained at 36.5±1°C with a temperature control unit (FHC) and a heating lamp (Skytron, Datichi Systems). Mean femoral arterial blood pressure (MFABP) (recorded in left femoral artery), regional spinal cord blood flow (rSCBF) at the lumbar level (L1), arterial blood gases, and pH were measured throughout the procedure in a parallel group of 5 animals (group B). A Centris 650 Macintosh computer and a Mac LAB/8 System (AD Instruments) equipped with an ETH-400 transducer amplifier were used for continuous acquisition and online analysis of data. MFABP was obtained from the left femoral artery with polyethylene (PE-10) tubing. We used a qualitative real-time measure of rSCBF by laser-Doppler flowmetry (PF2B, Perimed) with a 0.8-mm fiberoptic extension. The probe was affixed perpendicularly on the thinned and flattened posterior surface of vertebra L1 through a limited skin incision. Arterial blood gases (PaCO2 and PaO2) and pH were measured 10 minutes before ischemia and 10 minutes after the onset of reperfusion in 40-μL samples obtained from the left femoral artery via microhematocrit capillary tubes (Fisher Scientific) and a blood gas/pH analyzer (Corning 178, Ciba-Corning Diagnostics).

Evaluation of Neurobehavioral Outcome
Motor functions were graded according to the following previously published criteria16: (a) walking with hind limbs: 0, normal; 1, toes flat under body when walking but ataxia is present; 2, knuckle-walking; 3, movements in hind limbs but unable to walk; and 4, no movement, drags hind limbs; (b) placing/stepping reflex: 0, normal; 1, weak; and 2, no stepping. Each grade was obtained by adding the scores for a and b.

Histopathology
Animals were killed after 24 or 48 hours. The entire spine was harvested en bloc and immediately frozen in liquid N2. Ten-micrometer transverse sections were obtained at T5 through T7, T9 through L2, and L3 through L5 levels and stained with Luxol fast blue–hematoxylin and eosin.

Spinal Cord Arterial Blood Supply Determination/Carbon Black Perfusion Study
A surgical clip (*) hides the left internal mammary artery. The LCCA is visualized in the center of the field. The AA and the origin of the LSA are observed in the superior mediastinum. Surgical clips are placed on each of the vessels at the indicated sites to render the spinal cord ischemic. Bar=1 mm.

Figure 1. Operative view of the thoracotomy field showing the left common carotid artery (LCCA; center of the field), aortic arch (AA; arrowhead), and LSA (arrow). A surgical clip (*) hides the left internal mammary artery. LLSA (arrow). A surgical clip (*) hides the left internal mammary artery. LCCA is visualized in the center of the field. The AA and the origin of the LSA are observed in the superior mediastinum. Surgical clips are placed on each of the vessels at the indicated sites to render the spinal cord ischemic. Bar=1 mm.

Statistical Analysis
Parametric data were presented as mean±SD. ANOVA followed by Bonferroni and Tukey-Kramer tests was used to compare MFABP and rSCBF values, and paired Student’s t test was applied for blood
Physiological Parameters
Baseline MFABP was 62±11 and 56±18 mm Hg before ischemia and during reperfusion, respectively, which was somewhat lower (approximately 10 mm Hg) than values reported previously for anesthetized C57BL/6 mice; this was due to increased intrathoracic pressure and reduced venous return after thoracotomy. Clamping the aortic arch caused an initial drop in rSCBF, followed by a second decrease on clamping the LSA. rSCBF stabilized at 27±7% of baseline (Figure 3, top panel), which is somewhat higher than anticipated on the basis of MFABP of 10±5 mm Hg. Perhaps the laser-Doppler flow probe detected flow within deeper structures such as gray matter. After the aorta was reperfused, rSCBF returned to 76% of baseline within 10 minutes, and MFABP returned toward baseline (Figure 3). After surgery, there was a reduction in pH (P<0.05), a decrease in base excess (P<0.01), and a decrease in PacO2 (P<0.05), indicating the presence of metabolic acidosis and compensatory hyperventilation. A decrease in PaO2 (P<0.05) was caused by respiratory insufficiency after thoracotomy (Table).

Neurobehavioral Outcome
There were no deaths in group A (9 minutes of ischemia). In group B, 5 of 24 mice (21%) died before 24 hours, and generalized seizures were observed in 3. One died after prolonged anesthesia, and there was no obvious cause of death in the other. Bowel infarction was not detected by macroscopic examination.

In both groups A and B, animals developed acute paraplegia. Group A mice began to recover motor function between 0.5 and 6 hours.
Figure 4. Twenty-four animals were subjected to 11-minute spinal cord ischemia and reperfusion (see text). Motor function was evaluated by a motor deficit score (MDS), with a score of 6 indicating complete paraplegia. During the procedure, 5 animals died (○). Two animals showed nearly complete recovery (□, ◢), whereas in 2 others, the recovery was transient with complete paraplegia by 24 hours (◇, □). Most animals (15 of 24) survived with persistent paraplegia (◇).

3 and 24 hours, with complete recovery at 24 hours, whereas 80% of group B remained completely paralyzed with maximum deficits throughout the postoperative period (Figure 4). Two animals in group B showed transient recovery early but developed maximal and persistent deficit at 24 hours.

Histopathology
In both groups, histopathological changes were present in the spinal cord at lower thoracic and lumbar levels. T5 through T7 was always spared, whereas L1 through L5 was always affected. In group A, scattered “red neurons” appeared in dorsal horn and intermediate gray matter (Figure 5a). Ventral horns were affected to a lesser extent, with <10% of motor neurons remarkable for their eosinophilic cytoplasm. In group B, there were many red neurons in dorsal horn (with relative preservation of superficial lamina) and intermediate gray matter, and >50% of motor neurons were eosinophilic (Figure 5b). Parenchymal edema (vacuolar changes) was present in group B only. Two animals with restored motor function at 1 day showed milder histological findings consistent with those of group A. White matter was relatively spared in this model, with notable preservation of astrocytes and oligodendrocytes at 24 and 48 hours. In the most severe cases, axons surrounding gray matter showed degenerative changes.

Discussion
We showed that cross-clamping the aorta together with the LSA caused hind limb paralysis and neurological deficits in C57BL/6 mice. Both 9- and 11-minute occlusion damaged the lower thoracolumbar spinal cord. After 9 minutes of occlusion, motor dysfunction was severe, but it was reversible at 2 days. Pathology was limited to intermediate gray and dorsal horns predominantly, with preservation of anterior horns. After occlusion for 11 minutes, neurological deficits were severe, with persistent paraplegia. Pathology was as described above; additionally, more than half the number of anterior horn cells were pyknotic and showed eosinophilic cytoplasm. These changes correspond to the persistent motor deficit. The distribution of pathology in our model was similar to that described in humans and rodent models of spinal cord ischemia. Hence, the mouse may become useful to explore mechanisms of spinal cord injury.

The model described herein was adapted from the rat model described by LeMay et al., and to our knowledge this is the first description of a mouse spinal cord ischemia model. Stenonis developed the first model of spinal cord ischemia by ligating the descending thoracic aorta in the dog (see Reference 15). Since then, ischemic models have been described in dogs, pigs, and primates. However, these animals are expensive and surgery is time-consuming, whereas in mice (which are less expensive), a skilled surgeon can complete the procedure within 45 minutes. Numerous rat models have been developed, although none has gained wide acceptance because of poor reproducibility and relatively high mortality due to small-bowel infarcts and urinary bladder dilatation. The rabbit model has been widely used to test the effects of neuroprotective drugs and to examine the pathophysiology of spinal cord injury. However, little is known about the genome of rabbits, and transgenic rabbits have not yet been generated. The genome of mice, by contrast, has been intensively investigated, so that mutants containing gene deletions or extra copies of targeted genes have been generated, some of which may be relevant to ischemia, such as tissue plasminogen activator, superoxide dismutase, nitric oxide synthase, heat-shock proteins, and caspases to name a few. However, as in the rat, mice may develop bowel ischemia, which will limit their usefulness for evaluating the effect of neuroprotective drugs.

In humans, primates, pigs, dogs, and rats, cross-clamping of the aorta just distal to the LSA for 45 minutes often causes permanent neurological deficits. In these models, spinal cord blood flow (SCBF) is usually ≤15% to 20% at the lower thoracic and lumbar levels during the clamping peri-
Reproducible ischemia was achieved in the mouse only when both LSA and aorta were occluded, probably as a result of collateral channels arising from the subclavian-vertebral system. In fact, clamping the LSA provided an additional flow decrease and reduced distal mean aortic blood pressure (Figure 3), thereby reducing the cross-clamp time necessary to render the spinal cord ischemic.

During the development of this model, we were impressed that the magnitude of spinal cord injury was influenced by body weight, core temperature, perioperative plasma glucose levels, and choice of strain. Core temperature was measured with a rectal probe, which may of course differ from temperature in the spinal cord during the ischemic insult. Younger animals (body weight <18 g), fasting animals, and hypothermic animals seemed more resistant to spinal cord ischemia, as reported in other species, and age, plasma glucose levels, and temperature are important determinants of tissue outcome in brain ischemia as well.

129S6/SvEv mice seemed less susceptible to spinal cord ischemia than C57BL/6 mice, perhaps because of vascular or hemodynamic considerations, although carbon black perfusion did not identify gross differences in the vascular anatomy between strains. We observed wide variations in SCBF after occluding both the aorta and the LSA in 129S6/SvEv, despite severe (and equivalent) reductions in femoral artery blood pressure in both strains. We also noted that the occlusion times required to cause similar pathological deficits in 129S6/SvEv varied between investigators, despite side-by-side technical comparison. Fifteen minutes of occlusion with 1 experienced investigator achieved the same deficits as 11 minutes by another. Variability between investigators (not observed in C57BL/6 mice) could become problematic when 129S6/SvEv mice are used because mutant animals are generated most commonly from the latter strain.

Spinal cord ischemia remains an underappreciated clinical problem. Most cases develop after aortic aneurysm repair, traumatic aortic rupture, aortic dissection, and paraplegia/paraparesis developed most commonly after repair of the thoracic and thoracoabdominal aorta. This study was supported by a National Institute of Neurological Disorders and Stroke Project grant (NS10828) and a National Institutes of Health grant (1 R01 NS37411-01). Dr. Matsushita was supported by the Fondation pour la Recherche Medicale. Dr. Matsushita was supported by the Uehara Memorial Foundation and the Yamanouchi Foundation for Research on Metabolic Disorders. Dr. Hirt was supported by the Fondation Suisse pour les Bourses en Medecine et Biologie and by the Fondation SICPA.


Conclusion
In summary, this study demonstrated that spinal cord ischemia develops in the mouse with the use of a simple surgical technique. This model may become useful to examine spinal cord ischemia in mice expressing specific genetic mutations.

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Editorial Comment

Spinal cord ischemia and subsequent paraplegia is a significant perioperative complication of aortic surgery. This report describes the development of a mouse model of spinal cord ischemia that may assist investigators in studying the pathophysiology and treatment of this injury. Ischemia was produced by cross-clamping the aortic arch, left subclavian artery, and internal mammary artery. Nine or 11 minutes of occlusion followed by reperfusion was produced to assess hemodynamic, histopathological, and functional outcomes. During the ischemic period, spinal cord blood flow measured by laser Doppler decreased at the L1 level to approximately 27% of baseline. After 11 minutes of ischemia, mice showed extensive gray matter pathology and persistent paraplegia.

The authors correctly point out that mouse models of central nervous system injury are advantageous because genetic studies can be conducted to critically investigate the pathophysiology of the injury process. This model should therefore facilitate these investigations. The authors have also discussed the limitations of using laser-Doppler flowmetry to assess spinal cord ischemia. Because laser-Doppler most likely measures spinal cord blood flow within superficial white matter tracts, the hemodynamic consequences of this insult on gray matter structures remains to be determined.

The authors indicate that core temperature had a significant effect on spinal cord injury. Because rectal and spinal cord temperatures may differ during and after the ischemic insult, some precautions may have to be taken if pharmacological agents are tested in this model. Nevertheless, this interesting model should allow for the clarification of patterns and mechanisms of cellular injury after spinal cord ischemia.

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