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Rapid Ischemic Preconditioning with a Short Reperfusion Time Prevents Delayed Paraplegia in a Rabbit Model

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ABSTRACT

Background: Surgery for thoracic and thoracoabdominal aortic aneurysms can be complicated by a significant incidence of neurogenic deficits due to spinal cord ischemia. In this study, we investigated whether ischemic preconditioning (IPC) improves neurologic outcome in a rabbit model.

Methods: Forty rabbits underwent infrarenal aortic occlusion. The IPC group (n = 20) had 10 minutes of aortic occlusion to induce spinal cord ischemia, 40 minutes of reperfusion, and 30 minutes of ischemia, whereas the control group (n = 20) had only 30 minutes of ischemia. Tarlov scoring (0, paraplegia; 4, normal) was used to evaluate neurologic functions 7 days later, and spinal cord segments (L4-L6) were stained with hematoxylin and eosin for histologic evaluation.

Results: Complete paraplegia (grade 0) occurred in 15 (75%) of the 20 control animals, whereas in the IPC group, 13 (65%) of 20 animals were completely normal (grade 4) ($P < .05$).

Conclusion: IPC is beneficial for protecting against neurologic damage after transient aortic occlusion in a rabbit model; however, the protective mechanisms are not clear.

INTRODUCTION

Paraplegia is a catastrophic complication of thoracic and thoracoabdominal aortic surgery. The causes are multifactorial. The prevalence of paraplegia was reported to range from 0.9% to 40% after successful operations on the thoracic and thoracoabdominal aorta [Crawford 1981; Crawford 1986; Svensson 1993]. For protecting the spinal cord against ischemic injury, various methods, including partial bypass, temporary shunts for cerebrospinal fluid drainage, and local cooling of the spinal cord, have been developed [Pontius 1954; Carlson 1983; Symbas 1983; Svensson 1986; Safi 1996; Motoyoshi 2001]. *N*-Methyl-D-aspartate receptor antagonists, calcium channel blockers, free radical scavengers, methylprednisolone, intrathecal papaverine, local adenosine, and systemic adenosine A_{2A} agonist infusions have also been used for this purpose [Svensson 1993; Kannellopoulos 1997; Ross 2000; Cassada 2001; Wan 2001]; however, no method completely prevents the development of paraplegia.

Ischemic preconditioning (IPC) was first introduced in 1986. The phenomenon involves a brief episode of sublethal ischemia that does not produce physiological deficits and reperfusion that increases the tolerance to subsequent prolonged ischemic injury [Murry 1986]. The protective effects of IPC have been reported for the heart and brain [Garnier 1996; Barone 1998]. These effects have also been demonstrated in spinal cord ischemic injury in various animal studies [Matsuyama 1997; Munyao 1998; Zvara 1999; Abraham 2000].

We used a rabbit model to investigate whether ischemic tolerance could be induced in the spinal cord by rapid IPC with a brief aortic cross-clamping of the infrarenal abdominal aorta and reperfusion prior to a prolonged ischemic period. We also studied the effects of that tolerance on delayed paraplegia.

MATERIALS AND METHODS

We used 40 male New Zealand white rabbits (3.0 ± 0.2 kg) in this study. They were housed and maintained on a 12-hour light-dark cycle with food and water given ad libitum. All procedures were performed according to guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 5377-3, 1996). All animals had no neurologic deficits before undergoing anesthesia and the surgical procedure. The study was approved by the local ethics committee.

Surgical Procedure

After overnight fasting of the rabbit, an ear vein was used for intravenous drug and fluid administration. Premedication was provided by intravenous fentanyl (0.5 μg/kg). The rabbits were anesthetized with intravenous sodium pentobarbital (25 mg/kg) and intramuscular xylazine (2.5 mg/kg) and were allowed to spontaneously breathe 100% oxygen with a nonsealing face mask device. A rectal temperature probe was inserted, and the temperature was maintained at approximately 37°C with a circulating warm-water (37.5°C) heating pad beneath the animal's body. The median ear artery and the left femoral artery were cannulated with polyethylene (PE-60) catheters to record the mean proximal aortic pressure (MPAP) and the mean distal aortic pressure (MDAP). All

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pressures were monitored continuously with a pressure transducer (P23XL; Nihon Kohden, Tokyo, Japan) throughout the procedure. Pulse oximetry monitoring ensured oxygenation during that period.

Sodium heparin (100 U/kg) was administered intravenously and allowed to circulate for 3 minutes. The abdomen was entered retroperitoneally after preparation of the skin with iodine. The abdominal aorta was occluded with bulldog clamps just distal to the left renal artery and proximal to the aortoiliac bifurcation. Aortic clamping was confirmed by a reduction in the MDAP.

Experimental Procedure

Rabbits were randomized into the IPC group (n = 20) and the control group (n = 20). Rabbits in the IPC group underwent 10 minutes of ischemia, 40 minutes of reperfusion, and then 30 minutes of ischemia. Rabbits in the control group underwent only 30 minutes of ischemia; however, the rabbits were held for 50 minutes after aortic preparation before undergoing the ischemic insult so that both groups would have similar anesthesia times. All catheters were then removed, and the surgical wounds were closed. All animals were transferred to their cages for recovery, with food and fresh water given ad libitum. Seven days later, all rabbits were subjected to neurologic testing and then euthanized according to the rules of the Institutional Animal Care and Use Committee.

Neurologic Scoring

Seven days after the operation, all animals were evaluated by a single blinded observer according to a modified Tarlov scoring system: 0, atony; 1, slight movement; 2, sits with assistance; 3, sits alone; 4, weak hopping; 5, normal gait/hopping [Cassada 2001].

Histology

After the neurologic evaluation, all animals were anesthetized with 50 mg/kg intraperitoneal thiopental and 5% isoflurane. After direct left ventricular administration of 1.0 mL heparin, the animals were perfused with 1000 mL 0.9% normal saline solution, followed by 500 mL 10% buffered formalin. The spinal cords of the animals were removed, and the lumbar portions of the spinal cords were immersed in formalin for 48 hours. Spinal cord segments from the fourth to sixth lumbar vertebrae were embedded in paraffin, and serial transverse sections (5 μm) were obtained.

The slides were stained with hematoxylin and eosin and examined by light microscopy for evidence of cellular degeneration and necrosis. Morphologic evaluation of the spinal cords in the groups was performed in a blinded fashion.

Statistical Analysis

The statistical analyses were performed with SPSS for Windows (version 9.0; SPSS, Chicago, IL, USA). Data are expressed as the mean ± SEM. Neurologic deficit scores for the IPC and control groups were compared with the Mann-Whitney rank sum test. A *P* value <.05 was considered statistically significant.

RESULTS

There were no significant differences between the IPC and control groups with respect to heart rate, MPAP, MDAP, and rectal temperature values (Table). During cross-clamping of the aorta, the MDAP dropped to 16.0 ± 2.3 mm Hg in the IPC group and to 15.3 ± 2.1 mm Hg in the control group (difference not statistically significant). The 2 groups had similar arterial blood gas values at baseline and at the end of the last ischemic period.

When neurologic outcomes were evaluated 7 days after the operation, the incidence of ischemic injury was 7 (35%) of 20 animals in the IPC group (6 rabbits were grade 4, and 1 rabbit was grade 3) and 20 (100%) of 20 animals in the control group (15 rabbits were grade 0, three rabbits were grade 1, and two rabbits were grade 2). These differences in neurologic scores between the control and IPC rabbits were statistically significant (*P* < .001) (Figure 1).

Results of the blinded histologic study were correlated with the neurologic findings. There were no histopathologic abnormalities in the IPC group for the sections stained with hematoxylin and eosin. The cord specimens from animals with Tarlov scores of 3 and 4 showed only minimal cellular infiltrates in the gray matter, and there was good preservation of nerve cells (Figure 2A). The histological study of the spinal cord specimens from animals of the control group revealed severely injured neurons. Necrotic neurons either appeared as shrunken with a condensed perikaryon or displayed eosinophilic cytoplasm and pyknotic nuclei (Figure 2B).

DISCUSSION

Paraplegia is a major and usually irreversible complication of thoracic and thoracoabdominal aortic surgery. It results from the occlusion of segments of the aorta that may lead to spinal cord ischemia. The incidence of paraplegia after these surgical interventions is unpredictable, because the collateral blood flow to the spinal cord varies among individuals. The incidence may reach approximately 35% after extensive surgery for a thoracoabdominal aneurysm [Abraham 2000]. Despite the various surgical and pharmacologic approaches that have been taken, no single method has been shown to prevent paraplegia in this setting. IPC is supposed to play a role in improving ischemic tolerance of the heart by the adenosine A_1 receptor [Heurteaux 1995]. Although the protection mechanism of IPC has not been delineated for brain

Mean Proximal Aortic Pressures (MPAP), Mean Distal Aortic Pressures (MDAP), Heart Rates, and Rectal Temperatures during Ischemia in the Control and Ischemic Preconditioning (IPC) Groups

Group	MPAP, mm Hg	MDAP, mm Hg	Heart Rate, /min	Rectal Temperature, °C
Control	80.5 ± 5.3	15.3 ± 2.1	182.6 ± 5.4	38.4 ± 1.0
IPC	79.3 ± 5.5*	16.0 ± 2.3*	180.6 ± 6.0*	38.5 ± 2.2*

*P > .05. Data are presented as the mean ± SEM.

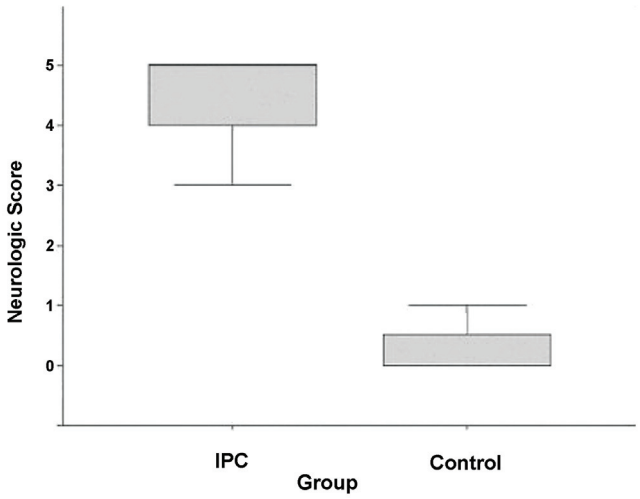
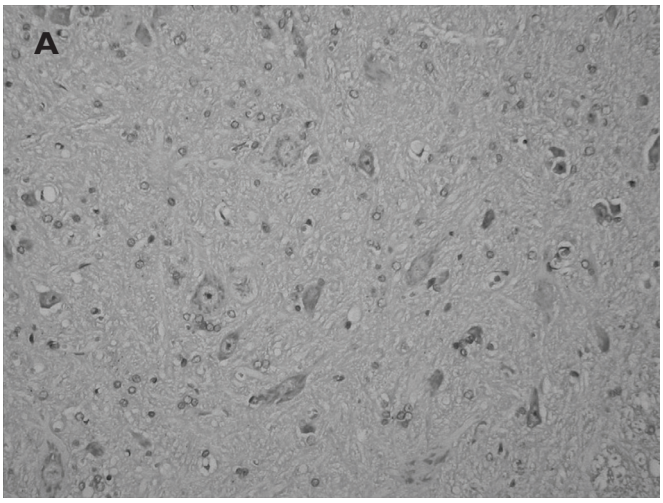


Figure 1. Box plot of neurologic outcome at 7 days after the procedure. The difference between the 2 groups (20 animals in each group) is significant ($P < .001$). The SEM is indicated. IPC indicates ischemic preconditioning.



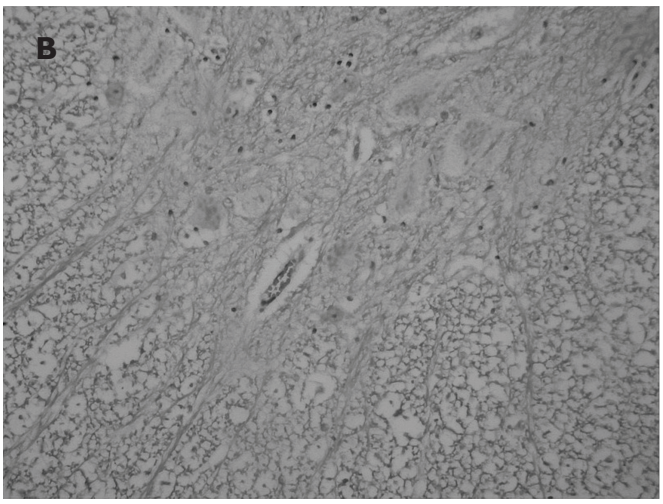


Figure 2. A, Representative photomicrograph of spinal cord sections of rabbits with Tarlov scores of 3 and 4 in the ischemic preconditioning (IPC) group. Only minimal cellular infiltrates in the gray matter and good preservation of nerve cells are seen (hematoxylin and eosin staining). B, Representative photomicrograph of spinal cord sections of animals from the control group that experienced paraplegia. The sections contained several injured neurons (hematoxylin and eosin staining). Necrotic neurons either appeared shrunken with a condensed perikaryon or displayed eosinophilic cytoplasm and pyknotic nuclei.

tissue, heat shock proteins (HSP), which can be produced in response to stress, are considered a "molecular chaperone" and maintain the configuration of proteins, thereby preventing degradation.

Matsuyama and associates demonstrated a protective role for IPC in spinal cord ischemia in a dog model, and they found a relationship between spinal cord protection and the HSP immunoreactivity of the spinal cord. They applied 20 minutes of IPC 48 hours before 60 minutes of a sublethal ischemic insult. The animals were evaluated 24 hours after the ischemic period. Although 3 of the 6 control animals were paraplegic, none of the 6 preconditioned animals had paraplegia. There were several limitations to that study. The sample size was small, and results obtained with the canine model were not completely applicable to humans [Cassada 2001]. Munyao et al [1998] studied rabbits that underwent 12.5 minutes of IPC 12 or 48 hours before 30 minutes of ischemic insult. The neurologic outcomes were better in the rabbits that underwent IPC 12 hours before the ischemic period than in the control rabbits and the rabbits that underwent IPC 48 hours before undergoing ischemia [Munyao 1998]. Increased HSP 70 and mRNA in motor neurons of the spinal cord were recorded in rabbits that were exposed to10 minutes of IPC 48 hours before undergoing 15 minutes of ischemia [Sakurai 1998]. IPC is thought to be neuroprotective in this way. A single neurologic evaluation was performed after7 days in this study [Sakurai 1998]. Abraham and colleagues concluded from their results with a rat model of spinal cord ischemia, which included 3 or 5 minutes of ischemia in the IPC groups, that the IPC technique might be useful as a practical approach in selected operations on the thoracoabdominal aorta in order to protect the medulla spinalis from ischemia [Abraham 2000].

Fan et al [1999] showed that rapid IPC may produce increased regional blood flow in the spinal cord and decrease the norepinephrine concentration after an irreversible ischemic period and thereby increase the ischemic tolerance of the spinal cord. In a rat model, Zvara and colleagues found that 3 minutes of aortic occlusion followed by 30 minutes of reperfusion and then 12 minutes of ischemia was protective for the spinal cord and was associated with less histologic damage [Zvara 1999].

The long-term efficacy of rapid IPC for brain protection is controversial. In animal models, Perez-Pinzon et al [1997] and Kato et al [1991] found that rapid IPC with 2 minutes of sublethal ischemia was not effective in preventing neuronal damage. In contrast, another group found that the efficacy of the rapid IPC lasted 7 days after ischemic insult

[Nakamura 2002]. We faced a similar issue with respect to spinal cord protection.

Kakimoto et al found that rapid IPC provides neuroprotection in the spinal cord at the 24-hour control, but this protection did not persist to the seventh-day control in a rabbit model of spinal cord ischemia [Kakimoto 2003]. However, the observation that the delayed protective effects of IPC, termed the "second window of protection," appear more than 24 hours after the initial insult has recently been investigated [Basaran 2005]. In the present study, we also demonstrated that rapid IPC with a short reperfusion interval (40 minutes) is an effective method for spinal cord neuroprotection 7 days after sublethal ischemia in the rabbit model. The neurologic function did not change with time; however, the mechanisms are not yet clear. We believe that further studies are necessary to understand the underlying mechanisms.

This model of spinal cord ischemia and protection has several limitations. Differences in spinal cord sensitivity to ischemia among species, variability in the blood supply, and the mechanism of neurologic injury affect any applications of the data obtained from these experiments. In our study, we showed that rapid IPC shortly before irreversible ischemia can protect patients from delayed paraplegia in operations resulting in spinal cord ischemia. Many operations that can cause spinal cord ischemia, such as thoracic and thoracoabdominal aortic surgery, are electively performed. That fact may allow the use of intraoperative neuroprotective measures, such as rapid IPC.

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