Exploring Spinal Cord Protection by Remote Ischemic Preconditioning: An Experimental Study

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Background. **Paraplegia is one of the most severe complications occurring after the repair of thoracic and thoracoabdominal aortic aneurysms. Remote ischemic preconditioning (RIPC) has been shown to mitigate neurologic damage, and this study assessed its efficacy in preventing spinal cord ischemia.**

Methods. **The study randomized 16 female pigs into an RIPC group (n = 8) and a control group (n = 8). The RIPC group underwent four cycles of 5-minute ischemia-reperfusion episodes by intermittent occlusion of the left iliac artery. All animals underwent systematic closure of the left subclavian artery and segmental arteries of the descending thoracic aorta to the level of diaphragm. Motor-evoked potential monitoring was performed in both hind limbs. Continuous electrocardiogram and hemodynamics were monitored, and pulmonary artery blood samples were collected. A neurologic assessment was performed 6 hours after the procedure. The thoracic and lumbar portions of the spinal cord were collected for histologic and immunohistochemical analysis.**

Results. **The bilateral motor-evoked potential amplitude responses were higher in the RIPC group ($p < 0.05$) than in the control group; the difference was detected already before spinal cord ischemia. Paraplegia occurred in 1 control animal. Immunohistochemical total scores of antioxidant response regulator nuclear factor erythroid 2-related factor 2 were better in the RIPC group (11.0; range, 8.5 to 14.0) than in the control group (5.2; range, 1.0 to 9.0; $p = 0.023$).**

Conclusions. **RIPC induces electrophysiologic changes in the central nervous system that may confer spinal cord protection extending the resistance to ischemia. The significantly higher nuclear factor erythroid 2-related factor 2 scores suggest better neuronal cell protection against oxidative stress in the RIPC group.**

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The incidence of neurologic complications varies between 10% and 20%, and permanent paraplegia occurs in 3% of reported series associated with thoracic and thoracoabdominal aortic aneurysm repairs. These adverse outcomes are related to inadequate spinal cord blood supply or insufficient protection against ischemia-reperfusion injury [1–4]. Different strategies, including improved surgical techniques and adjunctive procedures, such as perfusion strategies, cerebrospinal fluid drainage, pharmacotherapies, and hypothermia, have successfully reduced the incidence of postoperative spinal cord dysfunction [1–6]. In experimental spinal cord studies, Griepp and Griepp [7] showed a collateral network fed by large arteries both proximally and distally and by segmental vessels instead of a major artery arising from the level thoracic (Th) 7 to lumber (L) 1 [7]. The critical interference of this network results in spinal cord ischemia; alternatively, intraoperative sequential segmental artery sacrificing is thought to serve as an ischemic preconditioning stimulus for intraoperative spinal cord protection [7, 8].

The beneficial protective effects of remote ischemic preconditioning (RIPC), exposing nontarget tissue to an ischemic stimulus providing protection against subsequent more severe insult, have been widely studied in experimental and clinical settings [9, 10]. The underlying mechanisms of preconditioning are not fully understood. The signal is thought to spread systemically, consisting of a neuronal pathway, different biochemical messengers, or a combination of these mechanisms [11].

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Abbreviations and Acronyms

ASUB = (the left) subclavian artery
CTRL = control
IQR = interquartile range
L = lumbar
MEP = motor evoked potential
Nrf2 = nuclear factor erythroid 2-related factor 2
RIPC = remote ischemic preconditioning
ROS = reactive oxygen species
SA = segmental artery
Th = thoracic

Oxidative stress, imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses, and especially, lower production of ROS is considered one of the possible mechanisms behind the preconditioning phenomenon. Antioxidant response regulator nuclear factor erythroid 2-related factor (Nrf2) indicates the cellular redox status. The ischemia-reperfusion stimulus induces the translocation of Nrf2 from cytoplasm to nucleus binding to the antioxidant response element in DNA, consequently leading to the induction of various antioxidant enzymes [12]. Expression of caspase-3 is associated with the induction of DNA fragmentation and the activation of apoptosis [13].

To study spinal cord ischemia, we have developed an experimental porcine model mimicking thoracic aortic aneurysm procedures with the sacrifice of segmental arteries to identify methods of reducing spinal cord injury. In our previous experimental spinal cord study, we demonstrated enhanced motor-evoked potential (MEP) responses by RIPC [14]. The objectives of the present study were to confirm these findings and to study the underlying mechanisms of RIPC by intermittent iliac artery occlusion-reperfusion before spinal cord ischemia.

Material and Methods

The animals used in this study received humane care in accordance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Resource Council, National Academy Press, revised 1996). The University of Oulu Research Animal Care and Use Committee approved the research protocol.

Experimental Setup

Sealed envelopes were used to randomize 16 female pigs (7 to 8 weeks old) from a native stock to two groups, a RIPC group (n = 8) and a control group (n = 8). All animals underwent closure of the left subclavian artery and a systematic closure of the segmental arteries. In advance of closure procedures, the RIPC group underwent four cycles of 5-minute ischemia-reperfusion episodes by intermittent occlusion of the left iliac artery. The control group received a sham treatment.

Anesthesia Protocol

The animals were sedated with an intramuscular injection of ketamine (350 mg), midazolam (45 mg), and medetomidine (1.5 mg). Peripheral catheters were inserted into a vein of both ears. Anesthesia was induced with thiopental (25 to 125 μg) and fentanyl (0.5 mg). Cefuroxime (1.5 g) prophylaxis was administered preoperatively, as well as intramuscular glycopyrrolate (0.2 mg). The animals were intubated with a 6.0-mm cuffed endotracheal tube and ventilated with a ratio of 55% oxygen to 45% air mixture in the respirator. Anesthesia was maintained by a continuous infusion of fentanyl (0.025 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}) and ketamine (15 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}) as well as inhalation anesthesia of sevoflurane (1.0%), which was discontinued before baseline values were measured. One intravenous dose of rocuronium (0.1 mg/kg) was used for surgical relaxation at the beginning of the operation, more than 45 minutes before MEP monitoring.

Hemodynamic Monitoring and Biochemical Data

A 7F pulmonary artery thermodilution CritiCath Swan-Ganz catheter (Ohmeda GmbH, Erlangen, Germany) was inserted through the right femoral vein for invasive hemodynamic monitoring and blood sampling. An arterial catheter for pressure monitoring and blood sampling was placed on the right femoral artery. To monitor urine output and fluid balance, an 8Ch catheter was introduced to the urinary bladder. Rectal temperature and electrocardiogram were monitored throughout the experiment.

Blood gas values, pH, electrolytes, plasma ionized calcium, plasma lactate levels, hematocrit and hemoglobin levels (iSTAT Analyzer; iSTAT Corp, East Windsor, NJ) were measured at baseline, at the end of RIPC or sham treatment, at the end of the closure procedure, and at 60 and 90 minutes from the end of the last segmental artery ligation (Fig 1).

RIPC Procedure

The left iliac artery was exposed through the incision over the left iliac crest. The clamp was placed around the artery for RIPC. The artery was occluded for 5 minutes, followed by a 5-minute reperfusion period by releasing the clamp. This intermittent ischemia-reperfusion cycle was repeated four times. The reactive systemic hypertension and hypotension after occlusion and reperfusion were confirmed by monitoring arterial pressure and the electrocardiogram. The control group underwent the incision and exposure of the left iliac artery 40 minutes but without RIPC. Preconditioning was performed 15 minutes before spinal cord ischemia.

MEP Monitoring

The skull was exposed by a 7-cm midline longitudinal incision. Four wire leads for MEP stimulation were placed and secured over the parietal cortex, with 2 leads attached on the right side and 2 on the left side. The placement was