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

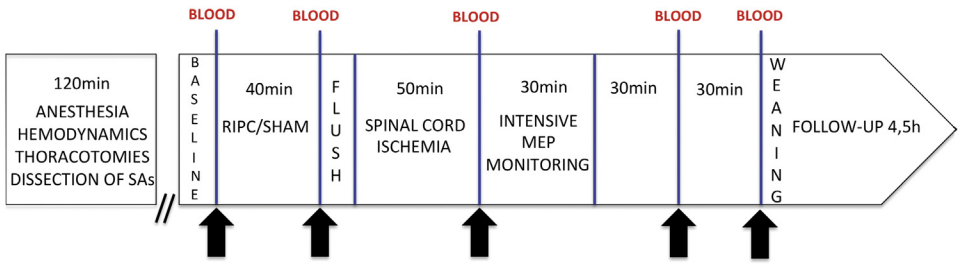

Fig 1. A simplified study protocol. Anesthesia induction, hemodynamic monitoring, thoracotomies, dissection of segmental arteries (SAs), and cranial procedures of motor-evoked potential (MEP) monitoring were performed before baseline measurements. A 15-minute interval (FLUSH) was conducted before spinal cord ischemia. (RIPC = remote ischemic preconditioning.)

based on sagittal and coronal sutures; 8 mm anterior and 8 mm posterior to the coronal suture and 10 mm lateral to the sagittal suture. Thereafter, 2 of the leads were connected to a TCS-1 electrical stimulator (Cadwell Inc, Kennewick, WA), and MEP stimulation was tested with different electrode selections.

Stainless steel needle electrodes were placed on both hind limbs (musculus tibialis anterior) to measure cortical motor nerve stimuli. Intraoperative neuromonitoring was performed by Cadwell Cascade Elite (Cadwell Inc) with Cadwell Cascade 2.6 software. A Cadwell TCS-1 constant voltage electrical stimulator was used for eliciting transcranial electrical MEPs. The stimulation electrode placements were described in the previous paragraph. The multipulse-stimulus characteristics were train length, 4; interstimulus interval, 2 ms;, and stimulus pulse width, 75 μs.

MEPs were monitored at baseline, at the end of RIPC or sham treatment, at closure procedures with 1-minute intervals, at 30-minutes postoperatively with 5-minute intervals, and at 60 minutes and 90 minutes from the end of the last segmental artery ligation. Peak-to-peak amplitude, baseline-to-peak amplitude, peak latency, onset latency, difference between peak and onset latency, and duration were analyzed (Fig 2).

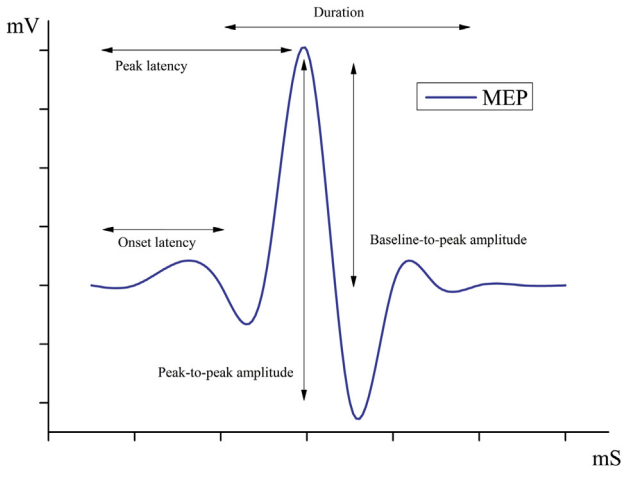

Fig 2. Motor evoked potential (MEP) response. The definitions of the onset latency, peak latency, peak-to-peak amplitude, baseline-to-peak amplitude, and duration are shown as lines with arrows.

Spinal Cord Ischemia

The fourth and seventh intercostal spaces were exposed through the left anterolateral thoracotomy for the dissection of the left subclavian artery and upper thoracic segmental arteries. In addition, an incision was made in the left 11th intercostal space to expose and dissect the rest of the segmental arteries to the level of the diaphragm.

After the surgical procedures, MEP baseline measurements were recorded under stable anesthetic and hemodynamic conditions. Within 15 minutes after the intervention, the permanent closure of the prepared left subclavian artery and a sequential permanent closure of the prepared segmental arteries were performed with 5-minute intervals simulating spinal cord ischemia. A 50% decrease of the MEP amplitudes compared with baseline values was considered indicative of critical ischemia.

Postoperative Management and Behavioral Evaluation

Surgical incisions were closed, and after a 90-minute period, the animals were weaned from mechanical ventilation, extubated, and transferred to a recovery room. Postoperative analgesia was maintained with an intramuscular injection of buprenorphine (6 μg/kg) after extubation until euthanasia.

Neurologic assessment was done 6 hours after the onset of spinal cord ischemia by assigning a Tarlov score of 0 to 4 [15]. The quantified assessment of motor function was performed as 0 = spastic paraplegia, no movements; 1 = paraparesis, slight movements; 2 = paraparesis, powerful movements in hind limbs, but not able to stand; 3 = able to stand but unable to walk; and 4 = full recovery, normal walking function. The maximum score of 4 indicates normal motor function and lower values reflect varying grades spinal cord damage.

Histopathologic Analysis and Immunohistochemistry

After the neurologic assessment, animals were euthanized using intravenous pentobarbital (90 mg/kg). The thoracic and lumbar segments of the spinal cord were harvested and immersed in 10% neutral formalin for fixation. The spinal cord was divided into five sections based on nerve roots Th1 to 3, Th4 to 6, Th7 to 9, Th10 to 13, and L1 to 4. The samples were sectioned at 6 μm in thickness and stained with hematoxylin and eosin. The exact preparation method is described in detail in our previous study [9]. Thereafter, the tissue sections from

each animal were screened and scored by an experienced neuropathologist unaware of the experimental design and the fate of individual animals.

The hematoxylin and eosin scoring system included the presence of edema (0 to 3), hemorrhage (0 to 2), neuron degeneration (0 and 1), and infarcted tissue (0 to 3). The maximum score for one region was 9, with a total score of 0 to 45.

The immunohistochemical stainings were performed on the spinal cord with five sections, as well. The scoring system for stainings, including Nrf2, was based on a semiquantitative protocol (0 = negative, 1 = positive, 2 = strongly positive, and 3 = very strongly positive; Fig 3). The scoring system for apoptosis-inducing protein caspase-3 was instead based on numeric calculations (0 = no stained motor neurons, 1 = 1 to 10 stained motor neurons, 2 = 10 to 20 stained motor neurons, and 3 = more than 20 stained motor neurons).

Statistical Analysis

IBM SPSS 22.0 software (IBM Corp, Armonk, NY) and SAS 9.3 software (SAS Institute, Inc, Cary, NC) were used for statistical analysis. Continuous and ordinal variables are expressed as the median with the interquartile range (IQR; 25th and 75th percentiles). Complete independence was assumed across all animals (by random statement). The repeatedly measured data were analyzed using a linear mixed model with animals fitted as random, and the covariance pattern was chosen according to the Akaike information criteria. Distribution of the variable was tested using the Shapiro-Wilks test of normality. The Student *t* test or Mann-Whitney *U* test was used to assess the *p* values between the variables of the study groups. Two-tailed significance levels are reported. Reported *p* values are as follows: *p* between groups (p_g) indicates a level of difference between the groups; *p* for time by group ($p_{t \times g}$) indicates behavior between the groups with time. A *p* value of less than 0.05 was considered statistically significant.

Owing to the individual conduction velocity of the spinal cord, the MEPs are presented as relative changes compared with the baseline values. The purpose of the statistical analysis for MEP responses was to explore the RIPC effects at different time points instead of performing exact global assessments over time.

Results

Comparability of Study Groups

All of the studied animals survived the entire closure protocol. The pigs in both study groups were a mean weight of 20.7 kg ($p = 0.990$). In summary, the study groups were balanced for metabolic and experimental variables throughout the experiment without clinically significant differences (Table 1).

Neurologic Evaluation

One animal in the control group died while weaning from the respirator, and the remaining 15 animals survived the experiment and lived for the entire 6-hour follow-up period. The neurologic assessment was performed at the end. The mean Tarlov score was 1.5 in the RIPC group and 1.1 in the control group ($p = 0.279$). Total paraplegia developed in 1 animal in the control group.

MEP Results

The MEP peak-to-peak amplitude responses in both hind limbs in the RIPC group were higher ($p < 0.05$) at several time points than in the control group. After RIPC had been performed at the postintervention time point, there was already a significant rise in peak-to-peak amplitudes in the left ($p = 0.021$) and in the right ($p = 0.022$) hind limb between the groups before spinal cord ischemia was induced. At this point, the control group did not show any changes, and a consistent group difference persisted until the end of the measurement series in both hind limbs. In the right nonischemic hind limb, the improved responses were also detected after occlusion of the left subclavian artery ($p = 0.040$) and 15 minutes postoperatively ($p = 0.049$), with statistically significant differences (Fig 4A and B). The baseline-to-peak amplitude responses demonstrated the difference with statistically significant responses in both hind limbs, in the left ($p = 0.021$) and in the right ($p = 0.012$) hind limb at the postintervention assessment, and after

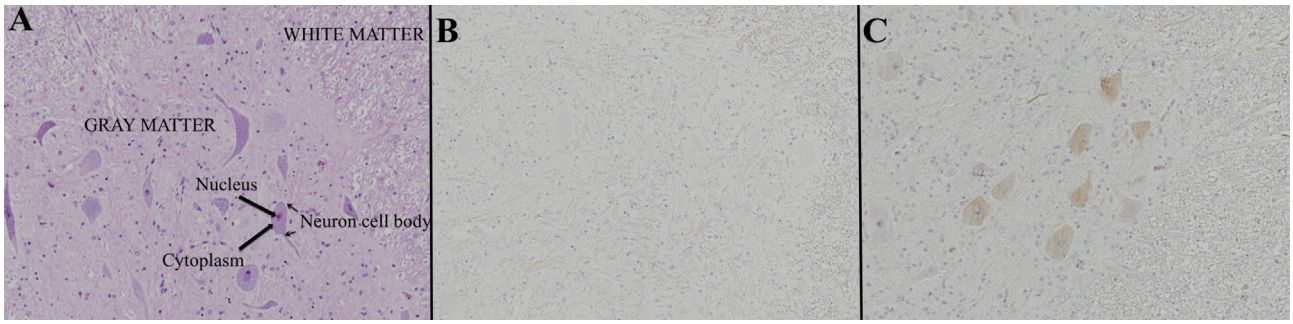

Fig 3. Representative photomicrographs (at original magnification ×40) of the spinal cord: (A) the anterior horn. Immunohistochemical stainings of nuclear factor erythroid 2-related factor 2 in the cytoplasm show the clear difference between the (B) control group (0 = negative) and (C) the remote ischemic preconditioning group (3 = very strongly positive).

Table 1. Experimental and Metabolic Data

Variable[a]	Baseline	After SA Closure				p_g	$p_{t\times g}$
		End of RIPC	End of SA	60 min	90 min		
Mean arterial pressure, mm Hg						0.51	0.44
RIPC	83 (79–92)	82 (73–88)	86 (79–99)	69 (62–83)	68 (63–83)		
Control	82 (80–90)	77 (72–79)	80 (76–89)	70 (64–80)	70 (62–73)		
Cardiac index, mL · min^{-1} · m^{-2}						0.52	0.31
RIPC	3.74 (3.13–4.45)	3.47 (2.72–3.94)	3.01 (2.58–3.31)	2.87 (2.36–3.25)	3.17 (2.58–3.40)		
Control	3.80 (3.54–4.29)	3.47 (3.29–4.63)	2.87 (2.69–3.27)	2.80 (2.60–3.61)	2.87 (2.59–3.98)		
Central venous pressure, mm Hg						0.17	0.32
RIPC	3 (2–4)	2 (2–3)	3 (2–4)	3 (2–3)	3 (2–4)		
Control	4 (3–5)	3 (2–3)	4 (3–5)	3 (2–4)	3 (2–4)		
Hemoglobin, g/L						.93	0.21
RIPC	97 (84–111)	92 (77–97)	92 (70–101)	91 (68–101)	80 (63–94)		
Control	88 (84–90)	87 (78–96)	85 (80–90)	85 (80–97)	85 (77–88)		
$Paco_2$, kPa						0.04[b]	0.12
RIPC	5.78 (5.59–6.07)	5.50 (5.43–5.61)	5.81 (5.48–6.07)	5.69 (5.37–5.80)	5.70 (5.16–5.80)		
Control	5.99 (5.56–6.30)	5.85 (5.64–6.32)	6.40 (5.79–6.97)[b]	5.95 (5.90–6.43)[b]	5.91 (5.69–6.23)		
Pao_2, kPa						0.05[b]	0.17
RIPC	40.7 (34.5–41.9)	41.7 (38.9–42.4)	39.6 (36.6–41.7)	43.4 (40.9–43.9)[c]	42.7 (39.5–44.4)[b]		
Control	37.7 (35.8–39.9)	38.8 (37.3–40.7)	36.7 (35.3–38.4)	39.0 (36.7–39.7)	40.0 (35.3–41.7)		
Oxygen consumption, mL · kg^{-1} · min^{-1}						0.17	0.63
RIPC	16.1 (12.6–18.2)	15.6 (12.5–17.0)	14.1 (12.4–15.0)	12.5 (12.3–14.6)	14.0 (11.7–16.5)		
Control	16.6 (14.5–18.9)	17.7 (13.4–19.7)	16.2 (13.3–18.6)	15.4 (13.3–17.6)	14.5 (14.4–16.0)		
Oxygen delivery, mL · kg^{-1} · min^{-1}						0.50	0.20
RIPC	52.1 (43.6–67.8)	42.1 (34.5–59.4)	42.1 (37.2–48.0)	37.4 (31.2–42.6)	37.9 (29.7–45.3)		
Control	52.8 (51.3–54.5)	49.4 (43.4–58.7)	42.6 (37.6–44.0)	41.9 (33.1–45.3)	44.1 (33.2–49.9)		
Rectal temperature, °C						0.04[b]	0.20
RIPC	37.0 (36.5–37.1)	36.7 (35.9–37.1)	36.4 (35.9–37.1)	36.0 (35.4–36.5)	36.3 (35.5–36.4)		
Control	38.0 (37.5–38.6)	38.1 (37.1–38.8)	37.9 (36.9–39.2)	37.9 (36.8–39.2)[b]	37.8 (36.6–38.8)[b]		
Blood temperature, °C						0.19	0.14
RIPC	37.1 (36.5–37.7)	36.9 (36.2–37.6)	36.8 (35.8–37.4)	36.9 (35.9–37.2)	36.8 (35.9–36.9)		
Control	38.6 (37.3–39.2)	38.1 (37.0–39.4)	38.3 (36.8–39.6)	38.5 (36.9–39.7)	38.2 (36.7–39.3)[b]		

[a] Values are shown as medians and 25th and 75th percentiles. RIPC, n = 8; control, n = 8. Data were analyzed using the Student *t* test/Mann-Whitney *U* test. [b] $p < 0.05$ at single time point. [c] $p < 0.005$ at single time point.

p_g = level of difference between the groups; $p_{t\times g}$ = behavior between the groups with time; RIPC = remote ischemic preconditioning; SA = segmental artery.

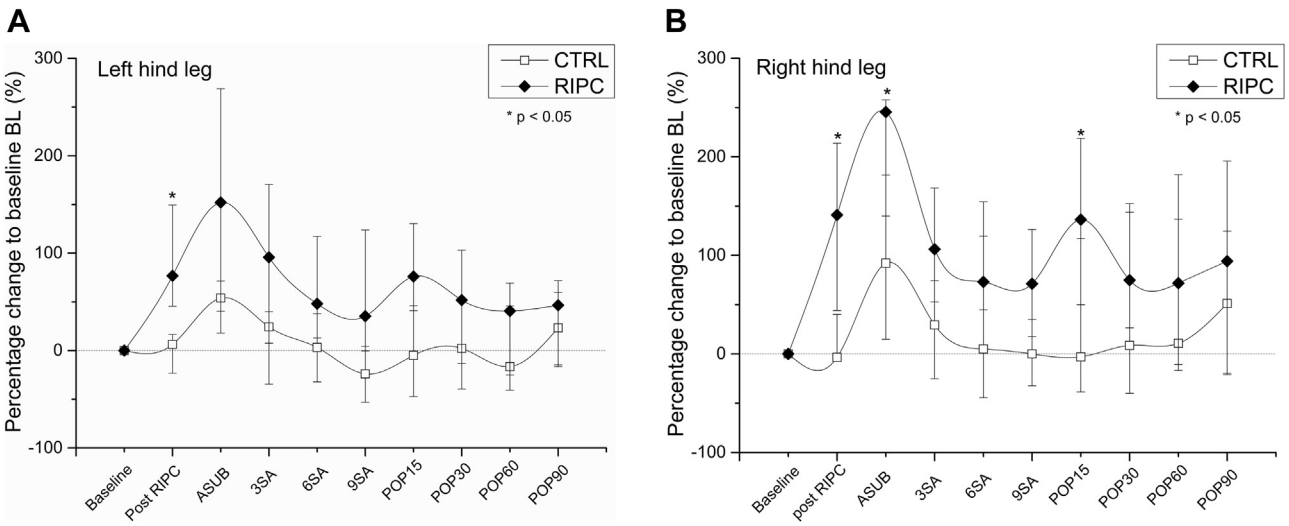

*Fig 4. Peak-to-peak amplitude changes in the (A) left hind limb and (B) right hind limb compared with the baseline (BL) values in the remote ischemic preconditioning (RIPC) group and the control (CTRL) group. The difference is statistically significant at the time point after RIPC (post RIPC). (B) In the right hind limb, statistically significant differences are also recorded after occlusion of the left subclavian artery (ASUB) and 15 minutes postoperatively. 3SA, 6SA, and 9SA stand for the specific time points with the number of occluded segmental arteries. POP indicates postoperative time points with minutes. Values are shown as medians and 25th and 75th percentiles. *$p < 0.05$ at single time point by Student t test/Mann-Whitney U test.*

occlusion of the left subclavian artery ($p = 0.046$) in the right hind limb.

The median time to 50% amplitude decrease in both study groups was 195 minutes in both hind limbs ($p = 0.798$). Peak latency, onset latency, and the difference between the peak and onset latency or duration were not statistically significant in the hind limbs at any time point.

Histopathologic and Immunohistochemical Findings

The sum of Nrf2 total scores was higher in the RIPC group (mean, 11.0; IQR, 8.5 to 14.0) compared with the control group (mean, 5.2; IQR, 1.0 to 9.0), with a statistically significant difference ($p = 0.023$). These scores were in favor of the RIPC group in all spinal cord sections (Table 2). The cytoplasmic staining reactions were detected in the anterior horns of the motor neurons. The expression of Nrf2 in the nucleus was negative in all samples. The immunohistochemical total scores with caspase-3 did not differ between the study groups ($p = 0.713$).

The mean sum of the histopathologic scores was 3.6 (IQR, 0.0 to 6.5) in the RIPC group and 2.8 (IQR, 1.0 to 4.5) in the control group ($p = 0.788$). The main findings were edema ($p = 0.868$), neuron degeneration ($p = 0.343$), and infarction score ($p = 0.064$). The hemorrhage score was similar between the groups ($p = 1.0$).

Comment

The current study underlines the experimental models that have demonstrated the use of direct or remote ischemic preconditioning is effective in reducing spinal cord ischemic injury [16–18]. The same improving effects were detected while measuring MEP responses in our previous experimental spinal cord study by RIPC, indicating higher conductivity of motor responses in the

Table 2. Immunohistochemistry

		Score[a]					
Immunostaining	Protocol	Th1–3	Th4–6	Th7–9	Th10–13	L1–4	Total
Caspase-3 (nucleus)	RIPC	1.6 (1.0–2.0)	1.8 (1.0–2.0)	1.9 (2.0–2.0)	1.4 (1.0–2.0)	2.0 (2.0–2.0)	8.6 (7.5–9.5)
	CTRL	2.3 (2.0–3.0)	1.6 (1.0–2.0)	1.5 (1.0–2.0)	1.6 (1.0–2.0)	2.0 (1.5–2.5)	9.0 (7.5–11.0)
	p value	0.069	0.765	0.135	0.333	1.000	0.713
Nrf2 (cytoplasm)	RIPC	2.1 (1.0–3.0)	2.4 (2.0–3.0)	2.4 (1.5–3.0)	2.1 (1.5–3.0)	2.0 (1.0–3.0)	11.0 (8.5–14.0)
	CTRL	0.6 (0.0–1.5)	1.1 (0.0–2.5)	1.5 (1.0–2.0)	1.1 (0.0–2.0)	0.9 (0.0–2.0)	5.2 (1.0–9.0)
	p Value	0.025[b]	0.074	0.077	0.092	0.057	0.023[b]

[a] Score is reported as mean (interquartile range; 25th and 75th percentiles). Data were analyzed by Student *t* test/Mann-Whitney *U* test. [b] $p < 0.05$.

CTRL = control (n = 8); L = lumbar; Nrf2 = nuclear factor erythroid 2-related factor 2; RIPC = remote ischemic preconditioning (n = 8); Th = thoracic.

spinal cord [14]. In our earlier studies we demonstrated a method of RIPC was useful in reducing neurologic damage to the central nervous system in ischemic brain injury associated with hypothermic circulatory arrest [9]. Exploring the protective mechanism, Donato and colleagues [19] used an experimental rabbit model of RIPC in cardioprotection to show the involvement of neuronal pathways.

In this study, the ultimate peak in the peak-to-peak amplitudes was observed after occlusion of the left subclavian artery in both study groups bilaterally. The closure itself might serve as an ischemic preconditioning, and therefore, the effect can be detected in the control group as well. In contrast, the occlusion of the left subclavian artery in clinical settings might cause a decrease in MEP responses. Hence, the significance of this first peak remains speculative. After occlusion of the left subclavian artery, the baseline values were gradually reached in the control group, whereas the improved MEP responses in the RIPC group lasted throughout the experiment, with a second peak 15 minutes postoperatively in both hind limbs, suggesting extended resistance to ischemia.

Nrf2 expression during ischemia is associated with neuronal cell protection [20]. Cytoplasmic Nrf2 staining reactions were clearly in favor of the RIPC group in all sections, signifying better neuronal cell protection against oxidative stress, as demonstrated by Dong and colleagues [21] as well.

At 6 hours postoperatively, the pigs were still recovering from the anesthesia, potentially decreasing their reactions to the assessment of motor function as indicated by low Tarlov scores. Moreover, a previous study showed that pigs have a chance to fully recover after 24 hours, despite the neurologic status at the early assessment [22]. Further studies with chronic models would verify the effectiveness of RIPC in spinal cord protection [4, 22].

Histopathologic findings with hematoxylin and eosin stainings were modest. Limitations of the study, including a short follow-up period as a result of Ethical Committee restrictions and a small group size, can partly explain these results. The rectal and blood temperatures remained higher in the control group throughout the experiments, and the possible effects on the histopathologic findings cannot be completely excluded. However, the same hyperthermia reaction of the control group was detected in our previous study without explaining the MEP results [14]. Mean arterial pressures were stable in both study groups during the entire protocol without interfering with the spinal cord perfusion pressure, which is crucial for normal spinal cord blood supply. Partial pressure of oxygen was higher and partial pressure of carbon dioxide was lower in the RIPC group postoperatively, indicating better oxygenation.

In conclusion, this study demonstrates that RIPC in advance of spinal cord ischemia induces significant electrophysiologic changes in the central nervous system that may confer spinal cord protection extending the resistance to ischemia. The high expression of Nrf2 suggests better neuronal cell protection against oxidative stress caused by spinal cord ischemia. RIPC performed for the iliac artery could provide a beneficial adjunctive protection strategy in thoracic aortic procedures. The exact mechanisms behind the preconditioning require further clarifications.

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INVITED COMMENTARY

Paraplegia resulting from spinal cord ischemia is a serious complication of thoracic and thoracoabdominal aortic repair. Many strategies, including reconstruction of segmental arteries, cerebrospinal fluid drainage, partial cardiopulmonary bypass for distal perfusion, elevating blood pressure, staged aortic operation, and the use of steroid, naloxone, and other protective drugs have been established. However, the possibility of paraplegia has not been reduced to nil. Herajärvi and colleagues [1] used transcranial motor-evoked potential (tc-MEP) monitoring to determine the efficacy of remote ischemic preconditioning, a unique technique to protect the spinal cord from ischemia, which might help to improve results of thoracic and thoracoabdominal aortic repair [1]. Myogenic tc-MEP recorded with an intramuscular electrode in the lower limb has long been used to detect spinal cord ischemia during thoracic and thoracoabdominal aortic repair. The electrical stimulus passes through the motor cortex, brain stem, spinal cord, peripheral nerve, and lower limb muscle, and MEP reflects any physiologic or structural change of any pathway. Precise analysis of spinal cord injury may require other narrow-range evoked-potential monitoring, including brain stem stimulation, lumbar epidural recording, and trans-intercostal nerve-evoked potentials [2]. Increased MEP amplitude results from various conditions, including elevating systemic or local temperature, increased arterial flow and pressure in the spinal cord supply, decreased intrathecal pressure, increased corticomotor excitability by somatosensory stimulation, and tetanic stimulation of the peripheral nerve.

However, most previous studies related to MEP monitoring during thoracic and thoracoabdominal aortic repair concerned decreased MEP. Significant MEP decrease was defined as an MEP amplitude to less than 50% or 25% of the baseline, and MEP increase was defined as a restoration of MEP followed by aortic intervention and an MEP decrease [3]. In this situation, what does MEP increase imply? Although the significance of an MEP increase after remote ischemic preconditioning remains unknown, it might imply conditions that are better protective for ischemic insults. A definitive percentage of MEP increase associated with protection of the spinal cord from ischemia–reperfusion injury should be determined.

Assessment of the motor function of animal hind limbs at 6 h postoperatively is limited because of the effects of anesthesia and other factors that might decrease the reaction to a stimulus. Limb function of animals including a rat, rabbit, dog, and pig often improves after 24 h despite low Tarlov scores 6 h postoperatively. Thus, we can draw conclusions only from chronic animal models with postoperative surveillance of 5–7 days that allow separation of paraplegic from non-paraplegic animals.

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