Remote ischemic preconditioning protects the spinal cord against ischemic insult: An experimental study in a porcine model

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ABSTRACT

Objective: Surgical repair of thoracoabdominal aneurysm jeopardizes the vascularization of the spinal cord, and therefore, despite improvement in surgical techniques, still carries the risk of paraplegia. This study aimed to demonstrate the possible protective effects of remote ischemic preconditioning (RIPC) on the preservation of spinal cord function after segmental artery (SA) occlusion.

Methods: Twenty piglets were randomized into the RIPC group (n = 10) and the control group (n = 10). The RIPC group underwent transient left hind limb ischemia before systematic left subclavian artery and SA occlusion at the level of the diaphragm. Motor-evoked potential (MEP) monitoring was performed from the hind limbs. Afterward, the thoracic and lumbar spinal cords were harvested and analyzed.

Results: The elevation of the MEP amplitude after RIPC was statistically significant, whereas amplitude was consistently decreased in the control group. Additionally, the onset latency was significantly shorter after RIPC during SA occlusion. The control group reached a 50% decrease of MEP amplitude in the right hind limb sooner than did the experimental group.

Conclusions: Remote ischemic preconditioning preserves spinal cord function after left subclavian artery and SA occlusion, as indicated by the MEP amplitudes. (J Thorac Cardiovasc Surg 2016;151:777-85)

Motor-evoked potential amplitude is higher after remote ischemic preconditioning.

Central Message

Remote ischemic preconditioning preserves spinal cord function after ischemic insult, as indicated by motor-evoked potentials.

Perspective

A significant risk of paraplegia still accompanies the surgical repair of thoracoabdominal aneurysms. Studies in a clinically relevant porcine model are important to verify the safety of and refine methods designed to prevent paraplegia. Remote ischemic preconditioning is one of the most studied topics in vascular medicine. This study revealed a protective effect of this method in the spinal cord.

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Permanent paraplegia or paraparesis after interruption of the spinal cord blood supply is a devastating complication of thoracoabdominal aortic repair procedures, although surgical techniques and the use of adjuncts such as cerebrospinal fluid drainage, hypothermia, and pharmacologic methods have improved outcomes remarkably in recent years.¹⁻⁴

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The segmental and nonsegmental vessels, primarily the subclavian and hypogastric arteries, are thought to form a vascular bed that is more crucial for spinal cord blood supply than is a single major artery arising from near the level of the diaphragm, as shown by Griep and colleagues⁵ in studies of the spinal cord blood supply. The threshold for spinal cord ischemia seems to be related to the number of segmental arteries (SAs) that are occluded and thereby cause a fatal interruption to the vascular bed supply.

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Abbreviations and Acronyms

MEP = motor-evoked potential
 RIPC = remote ischemic preconditioning
 SA = segmental artery

Two methods are available for spinal cord protection: maintenance of spinal cord perfusion during aortic clamping and neuroprotection during the time of ischemia and reperfusion.⁶ Attenuation of ischemic and reperfusion injuries can be achieved by priming neuronal tissue with a sublethal ischemic insult, a phenomenon known as remote ischemic preconditioning (RIPC). Thus, RIPC might be an effective way to prevent irreversible ischemic injury to the spinal cord during thoracoabdominal procedures.

The precise mechanism of RIPC has been the subject of intensive investigation. Currently, biochemical messengers, activation of the nerve pathways, or a combination of these, are considered to be protective mechanisms.⁷ Measuring motor-evoked potentials (MEPs) has been found to be a sophisticated and sensitive method for predicting ischemia before irreversible damage.^{2,8,9} A combination of these methods might provide valuable information about spinal cord protection in ischemia. Our objective was to investigate whether RIPC preserves spinal cord function after the interruption of the spinal cord blood supply in a porcine model of spinal cord ischemia with intraoperative measuring of MEPs.

METHODS

Experimental Setting

Twenty female juvenile pigs from a native stock (19–22 kg) were randomized into 2 groups using sealed envelopes: an RIPC group ($n = 10$) and a control group ($n = 10$). RIPC was performed in the RIPC group in advance of the segmental artery (SA) occlusion. The control group underwent a protocol identical to that in the RIPC group, excluding preconditioning.

Preoperative Management

All animals 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” (<http://www.nap.edu/catalog/5140.html>). The animals were in the same cage at least 7 days before experimentation, and the environment was kept nonstressful. The social nature of the animals was taken into consideration preoperatively. The Research Animal Care and Use Committee of the University of Oulu approved the study.

Anesthesia Protocol

Animals were sedated with an intramuscular injection of ketamine (350 mg), midazolam (45 mg), and medetomidine (1.5 mg). After endotracheal intubation, the subjects were ventilated with 50% oxygen and an end-tidal carbon dioxide concentration of 4.5% and 5.0%. Anesthesia was induced with fentanyl (50 $\mu\text{g}/\text{kg}$) and was maintained throughout the entire experiment by a continuous infusion of fentanyl (25 $\mu\text{g}/[\text{kg} \cdot \text{h}]$) and ketamine (15 $\text{mg}/[\text{kg} \cdot \text{h}]$). Ketamine or fentanyl has little or no effect on evoked potentials.^{10–12} Sevoflurane 1.0% was used as inhalation anesthesia, which was discontinued before baseline measurements. Rocuronium (0.1 mg/

kg) was used for relaxation and was not given after the baseline values were recorded.

Hemodynamic Monitoring

An arterial line for arterial pressure monitoring and blood sampling was placed in the right femoral artery. Mean arterial pressure was maintained at >60 mm Hg, and sodium chloride (0.9%) was infused if needed. A pulmonary artery thermodilution catheter (Criticath 7F; Ohmeda GmbH & Co, Erlangen, Germany) was introduced through the right femoral vein for blood sampling and invasive hemodynamic monitoring (Tables 1 and 2). Rectal and blood temperatures and electrocardiograms were monitored as well. A urinary catheter (10 Fr) was placed to monitor urine output, and the infused fluids were recorded to determine fluid balance.

Remote Ischemic Preconditioning

The RIPC design was identical to that of our previous study.¹³ The 9-cm-wide blood pressure cuff was placed around the left hind limb in the experimental group. The cuff was inflated to 250 mm Hg for 5 minutes, followed by a 5-minute deflation (reperfusion). The RIPC consisted of 4 inflation-deflation cycles, and the total duration of RIPC was 40 minutes. Control animals were observed for 40 minutes without RIPC.

Motor-Evoked Potentials Monitoring

A U-shaped incision was made in the scalp to reveal the skull, and the periosteum was removed. Four wire leads were attached to sterile stainless screws and screwed into the skull. The leads were placed 10 mm lateral to the sagittal suture, 2 on the left side (8 mm anterior and 8 mm posterior to the coronal suture), and 2 on the right side, respectively. The leads were connected to an electrical stimulator (Cadwell, TCS-1, Cadwell Laboratories, Inc, Kennewick, Wash). To measure cortical motor nerve stimuli, stainless-steel needle electrodes were placed through the skin in both hind limbs. An intraoperative neuromonitoring system, Cadwell Cascade Elite with Cascade software, version 2.6 (Microsoft Corporation, Redmond, Wash), was used in the study. A Cadwell TCS-1 constant-voltage electrical stimulator was used for eliciting transcranial electrical MEPs. The multi-pulse-stimulus characteristics were as follows: train length of 4, interstimulus interval of 2 milliseconds, and stimulus pulse width of 75 $\mu\text{-seconds}$.

The MEPs were recorded regularly at the baseline, after the RIPC or sham procedure, during the period of SA occlusion at 1-minute intervals, for a period of 30 minutes, after ischemia at 5-minute intervals, and at every 30 minutes throughout the 4-hour follow-up. The MEP data were analyzed with Cascade software, version 2.6. Peak-to-peak amplitude, onset latency, and difference between peak and onset latency were determined from the MEP data (Figure 1).

Surgical Procedures for Spinal Cord Ischemia

The fourth and seventh intercostal spaces were exposed from the left anterolateral thoracotomy for the dissection of the left subclavian artery and upper thoracic SAs. The rest of the SAs to the level of the diaphragm were exposed through the left, eleventh intercostal-space incision. Every animal had 9 SAs above the diaphragm.

After the surgery, stable anesthetic conditions and hemodynamics were confirmed before baseline MEP amplitude was recorded. Next, 15 minutes after RIPC (or sham), the prepared left subclavian artery and 9 SAs were ligated and occluded consecutively at 5-minute intervals. The decrease of 50% in MEP amplitude was considered to represent clinically relevant ischemia and was accomplished in all of the experimental animals used in this study.

Postoperative Management

Surgical incisions were closed in anatomic layers after SA ligations. After a 4-hour follow-up, the animals were extubated and transferred to a

TABLE 1. Experimental data

Variable	Baseline	Post RIPC	End of SA cutoff	1 h post op	2 h post op	4 h post op	P_g	P_{tg}
Heart rate (bpm)							.39	.047*
RIPC	98 (89-111)	125 (97-137)	81 (73-96)	76 (68-89)	86 (78-87)*	74 (70-87)		
Control	105 (88-120)	111 (91-118)	81 (76-86)	93 (86-107)	102 (94-108)	92 (86-93)		
MAP (mm Hg)							.97	.091
RIPC	89 (88-92)	70 (68-75)	78 (73-89)	74 (69-78)	73 (70-76)	64 (63-68)		
Control	73 (68-93)	75 (70-88)	83 (78-86)	74 (71-76)	72 (70-75)	64 (61-72)		
PA pressure (mm Hg)							.77/.33	.08/.62
RIPC	23 (20-24)/9 (7-11)	19 (16-23)/10 (8-11)	21 (20-23)/9 (6-10)	22 (18-23)/10 (5-10)	23 (22-25)/10 (8-11)	23 (21-25)/10 (9-11)		
Control	21 (17-23)/10 (9-10)	22 (20-25)/9 (9-10)	23 (19-25)/10 (8-11)	22 (18-24)/10 (9-11)	22 (21-23)/10 (9-11)	23 (21-27)/10 (9-11)		
Cardiac index $\text{mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$							.23	.58
RIPC	4.12 (3.81-4.36)	3.30 (3.10-3.57)	2.80 (2.22-3.01)	2.91 (2.36-3.13)	2.94 (2.58-3.18)	2.93 (2.64-3.15)		
Control	3.96 (3.49-4.81)	3.87 (3.43-4.08)	3.05 (2.55-3.20)	3.42 (2.91-3.46)	2.78 (2.60-3.97)	3.02 (2.23-3.52)		
Rectal temperature ($^{\circ}\text{C}$)							.18	.018*
RIPC	37.8 (37.5-38.9)	37.7 (37.1-38.2)	37.3 (37.2-38.2)	37.3 (37.1-38.4)	37.3 (37.1-37.6)*	37.1 (36.5-37.6)*		
Control	37.7 (37.5-39.0)	38.0 (37.3-39.1)	38.4 (37.0-38.9)	38.6 (37.4-39.1)	38.9 (37.9-39.1)	38.7 (37.5-39.5)		
Blood temperature ($^{\circ}\text{C}$)							.45	.61
RIPC	37.4 (36.8-38.8)	37.6 (36.6-39.2)	37.4 (36.6-39.2)	37.8 (36.7-39.4)	37.5 (36.9-38.6)	37.4 (36.9-38.1)*		
Control	37.5 (37.3-39.0)	37.8 (37.5-39.2)	38.2 (37.7-39.1)	38.7 (38.2-39.6)	39.1 (38.3-39.4)	38.7 (38.3-39.5)		
CVP (mm Hg)							.37	.39
RIPC	1.0 (1.0-2.0)	1.0 (0.0-1.0)	1.0 (1.0-2.0)	1.0 (0.0-2.0)	1.0 (0.0-2.0)	1.0 (0.0-2.0)		
Control	2.0 (0.50-2.5)	1.0 (0.50-1.5)	1.0 (1.0-3.0)	2.0 (1.0-2.5)	2.0 (1.0-2.5)	1.5 (0.0-3.0)		
O ₂ consumption $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$							.81	.48
RIPC	17.3 (14.9-18.1)	15.0 (13.9-17.7)	14.6 (13.0-18.7)	14.3 (13.2-16.4)	15.7 (13.4-16.9)	14.6 (12.8-15.2)		
Control	15.6 (15.0-17.3)	14.7 (12.0-18.1)	16.1 (15.1-18.4)	14.1 (13.3-17.9)	16.5 (14.7-17.8)	14.9 (14.7-16.5)		
O ₂ delivery $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$							.74	.88
RIPC	54.9 (54.3-55.8)	45.5 (44.2-50.1)	36.7 (31.7-42.7)	39.2 (32.2-42.2)	37.5 (33.6-44.4)	38.4 (36.6-40.3)		
Control	59.4 (47.6-63.4)	48.4 (46.1-58.0)	38.5 (34.0-39.9)	43.7 (34.5-47.0)	41.8 (35.0-47.5)	37.5 (31.3-49.1)		
O ₂ extraction mL/dL							.80	.43
RIPC	4.0 (3.7-4.2)	4.4 (4.2-4.9)	5.8 (5.4-6.3)	5.0 (4.6-5.2)	5.2 (5.0-5.7)	4.9 (4.5-5.3)		
Control	3.9 (3.5-4.6)	3.6 (3.1-4.5)	5.5 (5.1-5.9)	5.3 (4.1-5.7)	5.2 (4.7-6.1)	4.9 (4.2-7.5)		

RIPC n = 10. Control n = 8. Values are shown as medians and 25th and 75th percentiles. RIPC, Remote ischemic preconditioning; SA, segmental artery; *post op*, postoperatively; MAP, mean arterial pressure; PA, pulmonary artery; CVP, central venous pressure.

recovery room. Analgesia with buprenorphine, given at 6 $\mu\text{g}/\text{kg}$ intramuscularly was maintained until the end of the experiment. Neurologic assessment was performed precisely 24 hours after the onset of spinal cord ischemia, using a Tarlov score, as follows: 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; 4 = full recovery, normal walking function.^{14,15}

The animals were sacrificed with intravenous pentobarbital (60 mg/kg), and thoracic and lumbar spinal cords were harvested and immersed in 10% neutral formalin for 2 weeks. The spinal cord was sampled from 5 predetermined areas: thoracic vertebrae nerve roots¹⁴ 1-3, 4-6, 7-9, and 10-13 and lumbar vertebrae nerve roots⁵ 1-4. The length of thoracic or lumbar spinal column varied by 1 vertebra among the animals. These samples were then stained with a hematoxylin-eosin stain and scored blind by an experienced neuropathologist.

The sample preparation was performed with a method similar to the one we have used previously.¹³ Hematoxylin-eosin scoring was based on the presence of edema (0-3), hemorrhage (0-2), neuron degeneration (0-1), and the presence of infarcted tissue (0-3). The maximum value of a single

region was 9, and therefore all 5 regions analyzed had a total score range of 0 to 45.

Biochemical Data

Blood gas values, pH, electrolytes, plasma lactate levels, serum ionized calcium, glucose, hematocrit, and hemoglobin levels were measured using i-STAT Analyzer (i-STAT Corporation, East Windsor, NJ) at baseline, at 15 minutes after the end of RIPC, immediately after the ninth segmental artery occlusion, and 1 hour, 2 hours, and 4 hours after the ninth segmental artery occlusion (Figure 2).

Statistical Analysis

Statistical analysis was performed using SPSS (version 22.0; SPSS, Inc, Chicago, Ill) and SAS (version 9.2; SAS Institute, Cary, NC) statistical software packages. Continuous and ordinal variables are expressed as the median and 25th and 75th percentiles in Tables 1 and 2 and Figures 3-5. The repeated measurements were analyzed using a linear mixed model with animals fitted as random, and the best covariance pattern was chosen

TABLE 2. Metabolic data

Variable	Baseline	Post RIPC	End of SA cutoff	1 h post op	2 h post op	4 h post op	P_g	$P_{t \cdot g}$
pH							.62	.65
RIPC	7.48 (7.47-7.49)	7.49 (7.49-7.52)	7.47 (7.47-7.50)	7.45 (7.43-7.49)	7.45 (7.44-7.48)	7.47 (7.45-7.49)		
Control	7.49 (7.48-7.50)	7.48 (7.45-7.52)	7.46 (7.45-7.50)	7.47 (7.44-7.48)	7.47 (7.42-7.48)	7.45 (7.40-7.48)		
PaCO ₂ (kPa)							.92	.85
RIPC	5.75 (5.63-5.84)	5.52 (5.11-5.70)	5.45 (5.27-5.56)	5.66 (5.26-5.7)	5.34 (5.27-5.76)	5.42 (5.16-5.72)		
Control	5.64 (5.28-5.73)	5.41 (5.26-5.54)	5.55 (5.33-5.74)	5.48 (5.41-5.97)	5.30 (5.30-5.72)	5.47 (5.27-5.80)		
PaO ₂ (kPa)							.51	.71
RIPC	40.0 (35.9-42.1)	41.2 (38.4-43.5)	40 (39.5-41.4)	42.3 (40.2-43.7)	41.5 (41.4-42.8)	43.3 (38.8-44.9)		
Control	38.8 (37.2-39.9)	39.5 (38.8-44.9)	39.3 (36.9-41.4)	40.9 (38.2-41.7)	40.9 (36.7-42.8)	39.0 (31.7-43.1)		
Hematocrit (%)							.21	.53
RIPC	28.0 (26.0-29.0)	29.0 (28.0-30.0)*	28.0 (28.0-30.0)†	27.0 (24.0-28.0)	26.0 (25.0-28.0)	26.0 (25.0-26.0)		
Control	28.0 (26.0-31.0)	26.0 (24.5-27.0)	25.0 (24.5-27.5)	26.0 (23.0-27.5)	28.0 (24.0-28.0)	26.5 (23.0-28.0)		
Hemoglobin (g/L)							.20	.54
RIPC	95 (88-99)	99 (95-102)*	95 (95-102)†	92 (82-95)	88 (85-95)	88 (85-88)		
Control	95 (87-104)	88 (84-92)	85 (84-94)	88 (78-94)	95 (82-96)	90 (78-95)		
Venous glucose (mmol/L)							.82	.28
RIPC	6.0 (5.4-6.4)	5.7 (4.5-7.6)	3.6 (3.4-4.8)	4.4 (2.3-7.0)	4.7 (3.6-5.9)	3.3 (2.9-4.0)		
Control	5.5 (1.5-6.9)	5.3 (5.0-6.2)	4.2 (3.2-5.0)	2.6 (1.8-3.8)	5.0 (2.5-7.9)	3.9 (3.4-5.8)		
Venous lactate (mmol/L)							.30	.11
RIPC	0.81 (0.66-0.89)	0.59 (0.54-0.68)	0.79 (0.59-0.95)	1.05 (0.67-1.12)	0.81 (0.74-1.07)†	0.78 (0.67-0.86)		
Control	0.80 (0.60-0.99)	0.65 (0.45-1.01)	0.62 (0.52-0.85)	0.83 (0.74-0.98)	1.09 (0.98-1.48)	1.16 (1.04-1.67)		
Osmolality (mmol/kg)							.31	.65
RIPC	284 (282-286)	285 (285-289)	283 (282-288)	280 (276-285)	281 (280-287)	282 (277-291)		
Control	281 (278-284)	283 (281-286)	279 (279-282)	274 (272-279)	283 (277-289)	283 (278-289)		
Fluid balance (mL)‡							.43	.86
RIPC	600 (400-900)	600 (500-738)	850 (525-1025)	900 (600-1088)	1150 (725-1215)	1200 (825-1325)		
Control	500 (350-750)	500 (480-770)	600 (600-800)	800 (600-800)	850 (800-880)	1050 (900-1050)		

RIPC n = 10. Control n = 8. Values are shown as medians and 25th and 75th percentiles. RIPC, Remote ischemic preconditioning; SA, segmental artery; post op, postoperatively. * $P < .05$ at single timepoint. † $P > .05$ and $< .06$. ‡The fluid balance is presented as a cumulative amount of fluids within the follow-up. The urinary collection bag was drained at the baseline.

according to Akaike's information criteria. Complete independence was assumed across animals (by random statement). Reported P values are as follows: P between groups (P_g) indicates a level of difference between the groups, P time $\cdot$ group ($P_{t \cdot g}$) indicates behavior between the groups over time. The Student t test or Mann-Whitney U test was used, as appropriate, to assess the distribution of variables between the study groups. Two-tailed significance levels are reported; $P < .05$ was considered statistically significant.

Due to the individual conduction velocities of the spinal cord, MEPs are presented as relative changes from initial values. Hence, the P values of

multiple testing ($P_{t \cdot g}$ and P_g) are not reported on MEP values. Their purpose is not for an exact global assessment, but rather as a guide to recognize certain trends between groups at different timepoints.

RESULTS

Mortality

Two experiments were terminated because of intraoperative bleeding and excluded from the analysis. Four

FIGURE 1. Motor-evoked potential response. The figure is modeled from 1 pig. The definitions of the onset latency, peak latency, and peak-to-peak amplitude are shown as lines with arrows. MEP, Motor-evoked potential.

FIGURE 2. A simplified overview of the protocol. Baseline included anesthesia induction, invasive hemodynamic monitoring, thoracotomy, configuration of baseline MEP, and measurements. Before the start of spinal cord ischemia there was a 15-minute interval (FLUSH) for the data collection after RIPC and surgical preparation. Blood samples were taken at several timepoints, indicated by upward arrows. CTRL, Control; RIPC, remote ischemic preconditioning; SA, segmental artery.

animals were lost during observation, owing to respiratory problems. A total of 18 animals survived the 4-hour follow-up (10 in the RIPC group; 8 in the control group), and 14 animals survived at 24-hour follow-up (7 in each group).

Comparability of Study Groups

The mean weight of the RIPC group was 20.3 kg, and the control group 21.7 kg, respectively ($P = .083$). Blood ($P_g = .45$) and rectal ($P_g = .18$) temperatures were similar in the 2 groups. Hemoglobin did not differ between groups ($P_g = .20$). No difference was found in mean arterial pressure throughout the entire experiment ($P_g = .78$).

Experimental and metabolic data are summarized in Tables 1 and 2.

Motor-Evoked Potentials

The MEP amplitude change in the RIPC group demonstrated a tendency toward better function of the spinal cord after occlusion of the left subclavian artery and SAs (Figures 3 and 4). Strikingly, the RIPC itself caused an increase in MEP before the onset of spinal cord ischemia. Overall, the difference between groups reached statistical significance in the right hind limb recording at several points in time; left hind limb recordings showed a similar trend.

FIGURE 3. Peak-to-peak amplitude of right hind limb. Measurements are compared to the baseline value of 0. A clinically significant 50% decrease in the amplitude is marked with a dotted line. The amplitude change starts to diminish steadily after ligation of the left subclavian artery in the control group, whereas the amplitude is higher in the RIPC group after remote ischemic preconditioning. Values represent medians; error bars represent the interquartile range. RIPC, Remote ischemic preconditioning; CTRL, control; Subclavian l.sin, ligation of left subclavian artery; 5 SA, ligation of 5th segmental artery; 9 SA, ligation of 9th segmental artery; post op, postoperatively. * $P < .05$; ** $P < .01$; *** $P < .001$ at a single timepoint.

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FIGURE 4. Peak-to-peak amplitude in the left hind limb. Measurements are compared to a baseline value of 0. A clinically significant 50% decrease in the amplitude is marked with a dotted line. The difference is statistically significant after RIPC. Overall, the trend shows a slightly better preservation of the spinal cord in the RIPC group after ischemia. Values represent medians; error bars represent the interquartile range. *RIPC*, Remote ischemic preconditioning; *CTRL*, control; *Subclavian l.sin*, ligation of left subclavian artery; *5 SA*, ligation of 5th segmental artery; *9 SA*, ligation of 9th segmental artery; *post op*, postoperatively. * $P < .05$; $\nabla P < .06$ at a single timepoint.

Onset latency in the right hind limb was significantly shortened in the RIPC group after RIPC and lasted until the last SA occlusion (Figure 5). No statistically significant differences were found in the left hind limb. The differences between onset and peak latency were not significantly different in either limb.

The amplitude in the right hind limb in the RIPC group lasted throughout the 4-hour follow-up (345 minutes), whereas in the control group, the median time to a 50% amplitude decrease was 195 minutes (143-345 minutes), with a significant difference between groups ($P = .044$). The left hind limb recording did not show differences between the groups ($P = .271$).

Histology of Spinal Cord

The median sum of the histopathologic scores in the RIPC group was 9 (7.5-10.5) and also 9 in the control group (6-11), respectively ($P = .713$). The main findings were edema ($P = .410$) and hemorrhage ($P = .598$); the infarction score was similar between groups ($P = 1.0$). Neuron

degeneration did not differ significantly between the groups ($P = 1.0$).

Neurologic Evaluation

The 14 animals that survived through 24-hour observation were neurologically assessed. The RIPC group had a better mean Tarlov score (2.8) compared with the control group (1.8) after 24 hours of spinal cord ischemia, but with no significant differences ($P = .169$). One subject from the control group had a Tarlov score of 0; the minimum Tarlov score in the RIPC group was 2.

DISCUSSION

Despite widely used methods to protect the spinal cord during surgical repair of thoracic and thoracoabdominal aneurysms, a significant risk of paraplegia or paraparesis still exists.⁵ Studies using a porcine model seem to be essential in the investigation of the spinal cord blood supply and the reaction to ischemia.^{10,15} During the last decade, we have observed a neuroprotective effect of

FIGURE 5. Onset latency in the right hind limb. Measurements are compared to a baseline value of 0. The onset latency is significantly reduced in the RIPC group after intervention until the last segmental artery occlusion. Values represent medians; error bars represent the interquartile range. *RIPC*, Remote ischemic preconditioning; *CTRL*, control; *PR*, post-remote ischemic preconditioning; *Subclavian l.sin*, ligation of left subclavian artery; *5 SA*, ligation of 5th segmental artery; *9 SA*, ligation of 9th segmental artery; *post op*, postoperatively. * $P < .05$ at a single timepoint.

RIPC in ischemic brain injury associated with hypothermic circulatory arrest.^{13,16} However, evidence has been found against the protective effect, when the RIPC is performed in children suffering from chronic hypoxia.¹⁷ In this context, the chronic hypoxia may cover the effect

of RIPC. In the present study, we established an experimental model and observed that RIPC seems to have protective effects against spinal cord ischemia after occlusion of SAs simulating thoracoabdominal aortic aneurysm surgery (Figures 3 and 5).

FIGURE 6. Histologic findings from spinal cord. The presented photomicrographs are from ventral horns of the (A) control group porcine and (B) RIPC group porcine. Although the infarction score difference was not significant, the evident ischemia can be seen in the control group porcine. Spinal cord anterior horn shows ischemic, eosinophilic motor neurons (arrowheads), and vacuolar rarefaction of the background (asterisk), whereas in the RIPC group (B), stromal elements and motor neurons (arrow) are viable.

Recent studies have shown the benefit of RIPC after elective abdominal aorta aneurysm repair, in myocardial and kidney injury, as well as in lung and intestinal dysfunction.^{18,19} The neuroprotective effects of RIPC remain unknown, but preconditioning must be recognized by a cellular sensor that prepares cells for upcoming ischemic stress. Current consensus is that the RIPC neuroprotective effect is mediated by a number of substances, including bradykinin and adenosine. Hence, attenuation of oxidative stress and excitation of the anti-inflammatory signaling pathways seems to provide at least a partial explanation for RIPC protection against spinal cord ischemia.²⁰⁻²² In our previous study, we have found that RIPC reduces adherent leukocytes in cerebral tissue after hypothermic circulatory arrest.²³ This theory is supported by the large body of the evidence showing the role of inflammation in postischemic neuronal death, in both focal and global ischemia.²⁴

In the present study, RIPC improved MEP amplitude significantly in the right hind limb, and the response was more subdued in the left hind limb (Figures 3 and 4). In addition, the onset latency shortened immediately after RIPC in the right hind limb (Figure 5), and the phenomenon was not seen in the left hind limb. Histopathologic findings were minimal, as seen in the sum score, and injuries did not seem to prevent spinal cord function (Figure 6).

The results imply a considerably higher conductivity of motor responses in the spinal cord after RIPC. MEP amplitude is distinctly higher in the RIPC group after intervention, before the initiation of ischemia. The neurons of the spinal cord are better prepared for upcoming ischemia after RIPC, which might be explained by the above-mentioned mechanisms, although they have been studied more extensively in the context of myocardial ischemia.^{18,25-28}

In an experimental model by Donato et al.,²⁶ sectioning of spinal cord extinguished the protective effect of RIPC in the myocardium, indicating the involvement of neuronal afferent signaling. Therefore, we took extra precautions to not damage the femoral nerve while operating on the right femoral vessels. In summary, the mechanisms of RIPC include neurogenic and humoral pathways, and different routes are activated in various organs. However, the reason that MEP amplitude is at least twice as high in the RIPC group cannot be ascertained from our study.

The explanation for the weak left hind limb response might be the sacrifice of the left subclavian artery.¹⁵ The noncritical number of SAs has been shown^{15,29} to be 9 ± 3 . In addition to ligation of the 9 supra-diaphragmatic SAs, the left subclavian was occluded to cause sufficient ischemia to the spinal cord and to lead to interruption of left-side circulation.

This finding supports the theory of a vascular network being responsible for the spinal cord blood supply.^{5,30} Additional support comes from the fact that MEP

amplitudes in the control group are more decreased in the left hind limb. Another explanation may be that RIPC caused a reversible peripheral nerve injury to the left hind limb. Irreversible injury is not considered because the neurologic evaluation indicates faster recovery in the RIPC group after 24 hours.

Neurologic status was not significantly better in the RIPC group, although the trend in this group was favorable. The neurologic evaluation was made as early as at the first postoperative day when the animal may still be experiencing the effects of anesthesia and thus cause a bias in neurologic assessment. Moreover, the overall mortality was relatively high in present study. The overnight mortality tended to be higher in the RIPC animals compared with the control animals (3 vs 1). This difference can be explained by the small group size. On the other hand, the safety of the RIPC method has been demonstrated in many clinical studies.¹⁷⁻¹⁹

The mean arterial pressure did drop a clinically significant amount in the RIPC group after baseline. The drop is mainly explained as a cause of the transient limb ischemia, but the mean arterial pressure variation stayed inside the safety zone. Thereafter, the hemodynamic conditions remained stable throughout the experiment (Table 1). This stability is important because the spinal-cord perfusion pressure is dependent on the mean arterial pressure, and occlusion of noncritical SAs will become critical if the perfusion pressure decreases.²⁹

After the occlusion of the SAs, rectal temperature seemed to be higher in the control group, and as a natural reaction, the heart rate was higher (Table 1). Hyperthermia is known to reduce the latency and increase the conduction velocity of the MEPs; therefore, the differences in rectal temperature do not explain the result.¹¹ The higher core temperature might, however, explain the temporary rise in conduction and the reduction in the latency in the control group 2 hours postoperatively (Figures 3 and 5).

CONCLUSIONS

Our study shows that RIPC in a porcine model preserves spinal cord function after the SAs have been occluded. This study additionally supports the theory that the spinal cord has a vascular network that is responsible for the blood supply. The RIPC could provide a novel strategy to prevent spinal cord ischemic injury. The exact mechanisms of the RIPC neuroprotective effects warrant more studies.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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