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Ischaemic preconditioning reduces spinal cord injury in transient ischaemia

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Objective — Paraplegia remains a devastating complication after thoracic and thoracoabdominal aortic surgery for coarctations, dissections or aneurysms. Since the advent of ischaemic preconditioning of the myocardium, attention has been directed to the nervous system. This study was designed to evaluate the acute protective effect of ischaemic preconditioning on the spinal cord.

Medhods and results — Thirty-six New Zealand white rabbits were randomly assigned to one of three groups. The preconditioning group had 5 minutes of aortic occlusion, 25 minutes reperfusion and 20 minutes of ischaemia, whereas the controls had only 20 minutes of ischaemia. The sham group was anaesthetized and subjected to laparotomy without aortic occlusion. Physiological parameters and somatosensory evoked potentials were monitored during the experiment. Neurological outcome was clinically evaluated up to 48 hour after ischaemia and motor function was scored. Then the animals were sacrificed. Their spinal cord, abdominal aorta and its branches were removed and processed for histopathological examination. Histhopathological changes of the gray matter in the lumbosacral segments were scored from 0 to 6 according to a semi-quantitative scala.

The changes in amplitudes of evoked potentials during ischaemia and recovery periods were similar in preconditioning and control groups. The average motor function score was significantly higher in the preconditioning group than the control group at 24 and 48 hours after the ischaemic event ($p < 0.05$). Histological observations were consistent with the neurological findings. The histopathological scores in the control group and the preconditioning group were 3.2 (1.4-5.2) and 2.4 (0.8-4.4), respectively ($p < 0.05$).

Conclusions — The results suggest that ischaemic preconditioning reduces the spinal cord injury and improves neurological outcome in transient ischaemia in rabbits. This protective mechanism is rapidly invoked within only 25 minutes interval between the preconditioning stimulus and the ischaemic insult. *(Acta Cardiol 2002; 57(4): 279-285)*

Keywords: *ischaemic preconditioning – spinal cord – paraplegia – somatosensory evoked potentials.*

Introduction

Ischaemic spinal cord injury represents the main complication of thoracic and thoracoabdominal aortic surgery for coarctation, dissections or aneurysms. Deliberate hypotension, with the temporary clamping of the aorta may produce a critical reduction in spinal cord perfusion with a risk of irreversible ischaemic injury. Reperfusion may also cause numerous further negative effects when the ischaemia is severe and prolonged. To date, many techniques and adjunctive medications have been evaluated for efficacy in reducing this devastating complication. Some of them have met with varying success and are presently being used in several clinical settings, such as regional or systemic hypothermia[1], cerebrospinal fluid drainage[2], moni-

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toring of somatosensory evoked potentials (SEP) and reimplantation of major intercostal or lumbar arteries[3]. Despite their use, paraplegia remains a persistent clinical problem. The reported prevalence of neurological injury for these operations ranges up to 12 %. The risk of neurological injury is even higher in patients presenting with acute dissection or requiring emergency repair[4,5]. An easy and reliable surgical adjunct designed to reduce or eliminate the ischaemic spinal cord injury could be of great benefit in cardiovascular surgery.

Ischaemic preconditioning (IPC) is a powerful protective mechanism whereby brief ischaemic episodes increase the tissue resistance to a more prolonged ischaemic insult. This endogenous cellular phenomenon was first described in dogs by demonstrating that a series of short coronary occlusions followed by reperfusion protected the heart against the subsequent lethal ischaemia[6]. Currently, IPC is widely used during off-pump coronary surgery as a powerful protective mechanism against ischaemic injury of the myocardium.

Since the advent of IPC in the myocardium, attention has been directed to the nervous system. Many experiments were reported demonstrating the protective effect of the IPC in the brain[7,8]. Recently, a limited number of studies extending these observations to the spinal cord appeared in the literature[9-10]. In these studies, the reperfusion interval between the brief ischaemia and the subsequent potentially lethal ischaemia was several hours or days. The elevated synthesis of heat-shock proteins as a protective mechanism of IPC was speculated[7,10,12]. Although a long reperfusion interval is necessary to invoke this mechanism, some evidence suggests that shorter reperfusion intervals also provide neuroprotection[13]. Recently, the beneficial effects of rapid IPC with 30 min reperfusion interval was demonstrated in the brain[14]. Similarly, Fan and coworkers studied the potential benefits of IPC with a very short reperfusion interval (20 minutes) in the spinal cord[15]. The present study was designed to evaluate the protective effect of IPC with 25 minutes of reperfusion after the preconditioning stimulus in a rabbit model of spinal cord ischaemia.

Methods

Animal preparation

After approval of the study by the local Institutional Committee, 36 New Zealand white rabbits of both sexes, each weighing 2.3-3.5 kg (mean 2.9 kg.) were used in this experiment. All handling of the animals was in compliance with the European Convention on Animal Care. The animals were housed in individual cages with free access to water and laboratory chow. The room temperature was about 20-25°C. Anaesthesia was induced with intramuscular ketamine 50 mg/kg and xylazine 10 mg/kg as an initial dose, followed by ketamine 25 mg/kg fractionally as needed during the procedure. No animals received haemodynamic or ventilatory support. The animals were placed in a nose cone to breath oxygen at a rate of 3 l/min. Body temperature was monitored with a rectal probe and maintained close to 38°C using warming light. An intravenous catheter (24 gauge) was placed in an ear vein, and preoperatively cefazolin 30 mg/kg was given as a single dose. Maintenance fluid of 0.9% NaCl was infused at a rate of 20 ml/h during the procedure. A femoral artery was prepared for an arterial line with a catheter (24 gauge) and connected to a blood pressure/heart rate transducer and monitor (Hewlett-Packard 1495C). Percutaneous oxygen saturation was continuously monitored.

Experimental groups and surgical procedure

Rabbits were randomly allocated to three groups. Using aseptic techniques, the abdominal aorta was exposed and mobilised with median laparotomy. Each rabbit was anticoagulated with heparin 150 U/kg. Spinal cord ischaemia was induced with clamping the aorta just below the left renal vein with a bulldog clamp (FB328). A second similar clamp was placed distally, above the aortoiliac bifurcation for occluding iliac collateral circulation. The IPC group (n = 12) had 5 minutes of ischaemia, 25 minutes reperfusion and 20 minutes of ischaemia, whereas the controls (n = 12) had only 20 minutes of ischaemia. After the ischaemic period, the clamps were removed and restoration of the blood flow was confirmed visually. The abdomen was then closed. The sham group (n = 12) was anaesthetized and subjected to laparotomy and aortic dissection. The laparotomy incision was left open for 20 minutes corresponding to the spinal ischaemia period but the aorta was not clamped. This group of animals was used for assessment of the effects of the anaesthesia and the operation.

SEP recording

In order to assess the spinal ischaemia and neurologic function, somatosensory evoked potentials (SEP) were recorded before ischaemia, during ischaemia and the first 60 min of reperfusion. An active electrode was placed over the parietal region, whereas the reference electrode was on the nose. The sciatic nerve contralateral to the parietal electrode was stimulated by a bipolar surface electrode. Nihon Kohden Neuropack II plus (MEB 5000) was used for the recordings. The stimulus rate was 5/s with a duration of 0.2 ms and the stim-

ulus intensity was 3-6 mA. The bandpass filter range was 20 Hz to 1Kz. 256 responses were averaged.

Postoperative care and assessment

Venous and arterial lines were removed at the 60th min of reperfusion and all medication was stopped. The animals returned to their cages when they awakened from anaesthesia. The Crade manoeuvre was used to empty the bladders of paraplegic animals at least twice daily. Five animals (two rabbits originally assigned to the IPC group, 2 to the control group and 1 to the sham group) died during the experiment and were excluded from the study.

The rabbits were examined neurologically 24 and 48 hours after the operation and the spinal cord function was assessed by an independent observer. Bladder function was evaluated, and the motor function of the hindlimbs was scored according to the Tarlov scale[16]: 0, no movement; 1, trace movement; 2, sits with assistance; 3, sits alone; 4, weak hop; 5, normal motor function.

Histopathology

Animals were sacrificed with transcardiac 10% neutral formol injection under the intramuscular ketamine anaesthesia after the last neurological examination at 48 h post operation. The retroperitoneal region including the aorta and its branches were removed extensively and fixed in 10% formol solution. The spinal cord was then removed, immersed in the same fixative, and postfixed for two weeks before being set in paraffin blocks for sectioning. The abdominal aorta and its branches were examined for revealing possible thrombosis or embolic occlusion.

The L2 to S1 segments of each spinal cord were dissected in 5 pieces, tissue blocks taken from each piece were embedded in paraffin, and 4 micrometer sections were stained with haematoxylin and eosin. One section from each block was evaluated by an experienced histopathologist (RO). Using an ocular micrometer (Olympus occular eye X10), damaged neurons were counted and the histopathologic changes of the gray matter were scored on a 7-point scale[12]: 0, no lesion observed; 1, gray matter contained 1 to 5 eosinophilic neurons; 2, gray matter contained 5 to 10 eosinophilic neurons; 3, gray matter contained more than 10 eosinophilic neurons; 4, small infarction (less than one third of the gray matter area); 5, moderate infarction; (one third to one half of the gray matter area); 6, large infarction (more than half of the gray matter area). The scores from all the sections from each spinal cord were averaged to give a final score for an individual rabbit. This procedure was conducted in a blinded fashion.

Statistical analysis

The physiologic and haemodynamic parameters were expressed as means ± SD and compared among groups with one-way analysis of variance. Neurological outcome and histopathologic scores were expressed as medians and ranges.The Wilcoxon-Mann-Whitney U-test using the Bonferroni correction was performed on the data of the hindlimb motor scores and histopathological scores. The correlation was evaluated by Pearson bivariate analysis. SEP amplitudes measured during ischaemia and reperfusion periods were based on percent of baseline control values and expressed as means ± SD. Decrease and recovery of SEP amplitudes in the IPC group were compared with the values of control animals using the t-test and Bonferroni corrections. Fisher exact probability test was used in the analysis of bladder function. A p value less than 0.05 was considered significant.

Results

Physiological parameters

No difference in mean levels of heart rates, oxygen saturations or body temperatures was noted among the groups. The femoral artery blood pressure fell and remained near zero in the animals subjected to ischaemia during the clamping and became gradually normal within the first min of reperfusion.

SEP recordings

SEP recordings consistently showed a large negative peak (N1) with an average latency of 10 ± 3 ms, a positive peak (P1) with an average latency of 22 ± 4 ms and following negative peaks. The peaks were stable in sham operated animals during the procedure. Also, no change was noted during the preconditioning period (5 min ischaemia and 25 min reperfusion) in the IPC group. In ischaemic groups, N1-P1 amplitude progressively declined with an average of 10 ± 3 min after the aortic clamping. The amplitude changes in the preconditioning and the control groups were quite similar and the differences between the groups were not statistically significant. At the end of the ischaemic period, N1-P1 amplitude decreased to 58 ± 7 % of the pre-ischaemic baseline level in the IPC group whereas the decrease in the control group was to 55 ± 8 % of the baseline ($p > 0.05$). This was followed by a gradual return to 91 ± 7 % and 87 ± 9 % of the baseline after 60 min of reperfusion in the IPC and the control groups, respectively ($p > 0.05$). The typical changes in N1-P1 amplitudes are presented in Fig. 1.

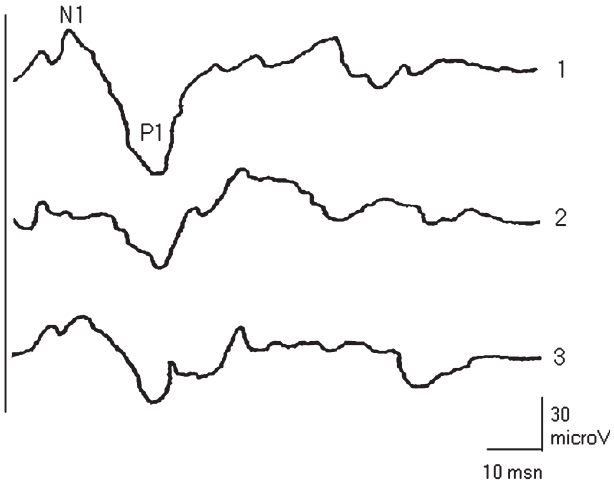

Fig. 1. – The typical changes in SEP traces during the procedure.

Neurological findings

There was no neurological dysfunction in sham operated animals and they exhibited full recovery after the procedure. Bladder function was retained to 48 h post ischaemia in 8 animals in the IPC group compared with 7 animals in the control group ($p > 0.05$).

The hindlimb motor scores after 24 and 48 h after operation in the IPC and the control groups are presented in Table 1. All of the animals in the IPC group exhibited score-3, score-4 or score-5 motor function whereas most animals in the control group (8/10) were evaluated as score-2 or score-3. Additional deterioration in motor scores was observed in 3 rabbits in the IPC group (1 animal from 3 to 2, 1 animal from 4 to 2 and 1 animal from 5 to 3) and in 2 rabbits in the control group (in both, from 3 to 2) within 24 to 48 h. The motor scores were significantly higher in the IPC group than in the control group at both 24 and 48 h after operation ($p < 0.05$).

Histopathological findings

Histological examination of the aorta and its branches was normal in all animals and revealed no thrombus formation.

Histological observations were consistent with the neurological outcome. Neurons in the fields of all sections were completely normal in appearance in sham operated animals. The animals with high motor scores exhibited an unaltered structure of gray matter or minimal necrosis in small patchy areas (Fig. 2). In contrast, severe neuronal damages were observed in the central gray matter of the lumbosacral segments which were detected primarily in the medial zone in the animals with poor motor scores (Fig. 3). The correlation between the histopathological scores and neurological findings are presented in Fig. 4. In comparison with the IPC group of animals, the control group showed significantly more damage in the central gray matter. The histopathological scores in the control group and the preconditioning group were 3.2 (1.4-5.2) and 2.4 (0.8-4.4), respectively ($p < 0.05$).

Discussion and conclusion

Since the introduction of IPC for the myocardium, many investigators focused on the effect of IPC in the nervous system. To date, many authors have reported that preconditioning the brain with sublethal ischaemia induced resistance to the subsequent potentially lethal period of ischaemia. This phenomenon of ischaemic tolerance of the brain has been well documented in both rats and gerbils. Much evidence suggests that the mechanism of the acquisition of ischaemic tolerance involves heat-shock protein (HSP) synthesis. In order to induce this effect, the interval between brief ischaemia and potentially lethal ischaemia must be greater than 1 or 2 days [7,8,17,18]. These observations were first extended to the spinal cord by Matsuyama

Table 1. – *Scores of hindlimb motor functions observed in the IPC and control groups 24 and 48 h after the procedure.*

Animal groups	n	MOTOR SCORE 0	1	2	3	4	5	Median motor score
IPC group								
24th hour	10	–	–	–	3	5	2	4 (3-5)*
48th hour	10	–	–	2	3	4	1	3 (2-5)*
Control group								
24th hour	10	–	1	3	5	1	–	3 (1-4)
48th hour	10	–	1	5	3	1	–	2 (1-4)

* Significantly different from the control group ($p < 0.05$).

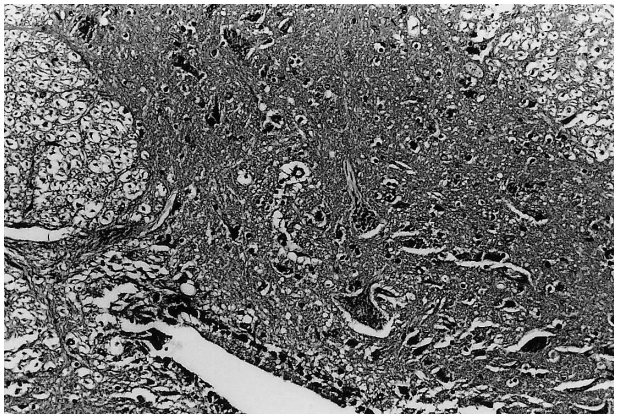

Fig. 2. – Spinal cord lumbosacral segment. Neuronal injury characterized by pyknosis and karyorrhexis (IPC group, histopathological score 2, motor function score 4, *100 haematoxylin and eosin).

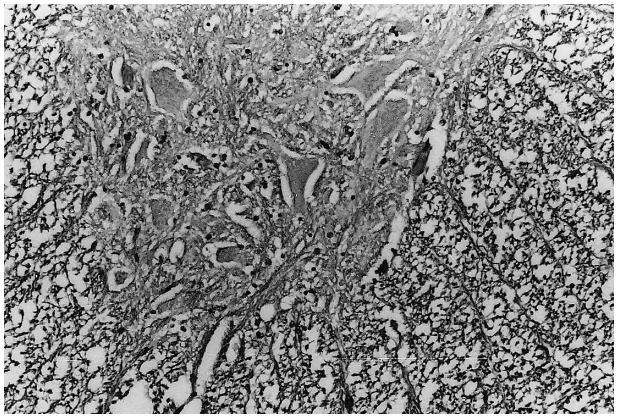

Fig. 3. – Spinal cord lumbosacral segment. Large necrosis in central gray matter characterized by the neurons with blurred cytoplasmic border and karyolysis (Control group, histopathological score 6, motor function score 1. *100 haematoxylin and eosin).

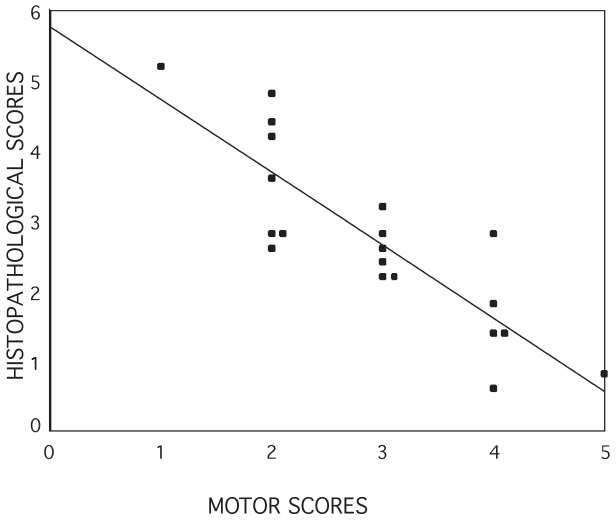

Fig. 4. – The significant correlation between the motor function scores and the histopathological observations in the studied groups of animals (n = 20; r = – 853; p < 0.01).

and co-authors[9]. This was followed by a very limited number of studies with conflicting results[10,11,12]. A recent study which was performed by de Haan and co-workers demonstrated no neurological or histological benefit of IPC in the rabbit spinal cord, despite the increased HSP levels after the preconditioning stimulus[12].

The results of the present study suggest that IPC reduces spinal cord injury and preserves neurological function in a rabbit model of spinal cord ischaemia. In addition, this protective mechanism is invoked acutely after a 25 min reperfusion interval between the preconditioning stimulus and the subsequent ischaemic event. This protection has been observed in both neurological and histopathological outcome. This study documents an acute benefit of IPC and differs markedly from the previous works. Matsuyama and associates demonstrated the protective effect of IPC in a canine spinal cord injury. They measured heat shock protein synthesis and found a positive correlation between HSP and neurological protection[9]. Sakurai and associates have also confirmed that IPC increases the HSP70 gene expression and protects the motor neuron cells against subsequent lethal ischaemia in a rabbit model of spinal cord ischaemia[10]. However, the reperfusion interval between the IPC and subsequent ischaemic insult was 48 hours in both studies. These models with such long reperfusion intervals require two individual interventions and may not be practical for clinical applications. They are not as applicable as the present study to the clinical settings of cardiovascular surgery. This experiment showed an acute benefit of IPC and significant neuroprotection with very short reperfusion interval. We think this model is relevant to the clinical situation that may be applied when one wishes to increase the tolerance of the spinal cord during the operation.

Although a role of the stress response is suggested, the exact mechanism of acquisition of ischaemic tolerance is currently unknown. Our data clearly demonstrated that the protective mechanism is acutely invoked after the preconditioning stimulus, suggesting that additional mechanisms other than HSP may be involved. Fun and associates showed a slight increase of norepinefrine and the regional blood flow in the spinal cord acutely after the preconditioning stimulus in a rabbit model. However, the neurological or histopathological outcome was not studied in their work[15]. Considering the cardioprotective efficacy of IPC, one can speculate that IPC may increase the release of adenosine and the stumulation of A1 receptors. In this initial experiment, we did not investigate the norepinefrine, adenosin, or any particular agent as a mediator of the protection. It should be mentioned that occlusion of the rabbit abdominal aorta produces

transient ischaemia not only in the spinal cord but also in some other parts of the body such as lower extremities, and the resulting biochemical changes may also be involved in the protective effect of IPC. These points require further study.

The occlusion of the rabbit abdominal aorta is a reliable model for systematically and rapidly observing the protective effects of investigated agents or techniques on the spinal cord ischaemia and reperfusion injury. However, blood flow may remain in some animals because of incomplete aortic occlusion due to technical insufficiency or individual variations in residual collateral circulation[19,20]. In the present study, an open surgical technique was used to access the abdominal aorta and a second clamp was applied distally for occluding the iliac collateral circulation. The open technique has permitted a visual and manual check of the occlusion of the vessel and collapse in the occluded segment. The SEP changes as an indicator of a significant decrease in regional spinal blood flow were well-documented in some previous studies[21,22]. Currently, most authors believe that SEP recordings reliably reflect the spinal cord function and it is widely used as a monitor of spinal cord ischaemia in clinical and experimental settings[15,23]. Monitoring of SEP is safe, easy and it does not prolong the operative procedure. Thus, we preferred SEP monitoring for ensuring duration and effectiveness of the global spinal ischaemia and identification of the animals that had sufficient collateral blood flow. It is known that a decrease of 15-20 % in amplitude can be taken as a criteria of significant SEP changes[24]. In the present study, SEP amplitudes decreased to about 55% of their baseline in all ischaemic animals with minimal individual variations. Observation of the similar SEP changes may indicate the similar collateral blood flow properties in the studied groups of animals. The possible effects of the anaesthesia and the operation on SEP recordings was ruled out by the evaluation of the sham group in the present study.

While some authors attempt to define the SEP recovery during early reperfusion as a predictor of neurological outcome, some articles have documented some examples where SEP did not accurately predict postoperative deficits, particularly motor deficits[25,26]. In the present study, although the difference in recovery of SEP amplitudes did not attain statistical significance ($p > 0.05$), we observed significantly better neurological outcomes in the IPC group ($p < 0.05$).

It is known that deterioration in neurological function after transient spinal cord ischaemia in rabbits usually worsens 24-48 h after injury. In this study, 3 rabbits in the IPC group and 2 rabbits in the control group showed additional motor deterioration within 24-48 h after the ischaemic insult. Although the mechanisms to account for this progressive deterioration remain uncertain, some investigators have suggested that cytotoxic substances such as free radicals, excitotoxins, proteases, or arachidonic acid metabolites found in damaged neural tissue are responsible[27,28].

In conclusion, the results of this experiment suggest that IPC has a significant, protective effect on spinal cord ischaemia and reperfusion injury. It reduces the spinal cord injury and improves neurological outcome in transient ischaemia in rabbits. Additionally, this endogenous protective mechanism is invoked within a very short reperfusion interval (25 min) after the preconditioning stumulus. However, the exact mechanism of the acquisition of ischaemic tolerance remains uncertain and the results of an animal study such as this may not be fully reproducible in humans. Further studies are needed to define the biochemical aspects of these events.

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