

# Does Ischemic Preconditioning Reduce Ischemia-Reperfusion Injury in Rabbit Spinal Cord?

## İSKEMİK PRECONTİNİONİNG TAVŞAN MEDULLA SPİNALİSİNDE İSKEMİ REPERFÜZYON HASARINI AZALTIR MI?

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### Summary

**Objective:** We investigated the effect of ischemic preconditioning on ischemic spinal cord injury in a rabbit model.

**Material and Methods:** Twenty-four rabbits were divided into four equal groups, group I (control), group II (ischemia), group III (preconditioning), group IV (ischemic preconditioning). Spinal cord ischemia was induced by clamping the aorta both below the left renal artery and above the aortic bifurcation. The spinal cord function of all subjects were assessed clinically 48 hours after aortic declamping. Spinal cord samples were taken to evaluate the histopathological changes.

**Results:** The rabbits in control group (group I) showed no neurologic deficit. Paraplegia (score 0) developed in all rabbits in the ischemia group (group II). Of group III (preconditioning group), 5 subjects were Tarlov scale 4, whereas one subject was Tarlov scale 3. Ischemic preconditioning subjects in groups IV demonstrated significant reduction of motor disfunction. There were no histopathologic abnormalities in hematoxylin-eosin-stained specimens of sham-operated rabbits. In group IV, the cords of subjects with Tarlov's scale 3 and 4 showed only minimal cellular infiltrates in the gray matter, and there was remarkable preservation of nerve cells. In histologic examination of paraplegic subjects, spinal cord samples obviated neuronal injury.

**Conclusion:** Our study showed that ischemic preconditioning may enhance the tolerance of the spinal cord against irreversible ischemia, and protect the neurofunction of the spinal cord.

**Key Words:** Ischemic preconditioning, Spinal cord

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### Özet

**Amaç:** Bu çalışmada biz medulla spinalis iskemisi üzerine iskemik önşartlamanın etkisini bir tavşan modelinde araştırdık.

**Gereç ve Yöntemler:** Yirmidört tavşan, grup I (kontrol), grup II (iskemi), grup III (önşartlama), grup IV (iskemik önşartlama) olarak dört eşit gruba bölündü. Medulla spinalis iskemisi aortik bifurkasyonun üzerinden ve renal arterlerin altından aortanın klemplenmesi ile sağlandı. Aortik klemplerin serbestleştirilmesinden 48 saat sonra tüm deneklerin medulla spinalis fonksiyonları değerlendirildi. Histopatolojik değerlendirme için medulla spinalis örnekleri alındı.

**Bulgular:** Kontrol grubunda (grup I) hiç norolojik bulgu yoktu. İskemi grubundaki (grup II) tüm tavşanlarda parapleji gelişti. Grup III (önşartlama grubu)'de 5 denek Tarlov skalası 4, 1 denekte ise 3 olarak bulundu. İskemik önşartlama koruma yapılan deneklerin (grup IV) motor bozukluklarında önemli azalma görüldü. Kontrol grubundaki tavşanların hematoksin-eosinle boyanan kesitlerinde histopatolojik anormallik yoktu. Grup IV'ün Tarlov skoru 3 ve 4 olanlarında gri matriksde minimal hücre infiltrasyonu ile iyi korunmuş sinir hücreleri vardı. Parapleji gelişen deneklerin medulla spinalis örnekleri şiddetli hasara uğramış nöronlar içeriyordu.

**Sonuç:** Bizim çalışmamızın sonucunda iskemik önşartlama iskemiyeye karşı medulla spinalisin toleransını artırabileceği ve medulla spinalisin nörofonksiyonlarını koruyabileceği görülmektedir.

**Anahtar Kelimeler:** İskemik önşartlama, Medulla spinalis

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Spinal cord injury after a successful operation on the thoracic aorta is a disastrous and unpredictable complication. The reported incidences of paraplegia is 0.9% to 40% for the thoracic aortic surgery (1-2). In an attempt to prevent this complication, various methods of spinal cord protection have been sug-

gested, including temporary shunts or partial bypass (3-4), hypothermia (5-6), drainage of cerebrospinal fluid (7), and pharmacologic measures (8-10). Regardless of the surgical technique or method of spinal cord protection used, none of these modalities has totally prevented the development of paraplegia (11).

Ischemic preconditioning is an endogenous cellular protective mechanism whereby brief, noninjurious periods of ischemia render a tissue more resistant to a subsequent, more prolonged ischemic insult. More recently, it has been shown that ischemic preconditioning provided substantial neuroprotection (12). Numerous studies have confirmed the neuroprotective efficiency of ischemic preconditioning in animal models (12-14). Fan et al. (15) reported that 5 minutes of ischemic preconditioning may increase the regional spinal cord blood flow in rabbits. We hypothesized that ischemic preconditioning of the spinal cord would reduce neurologic injury after experimental aortic occlusion in rabbits and that this improved neurologic benefit could be induced acutely after a short reperfusion interval separating the ischemic preconditioning and the ischemic insult.

## Material and Methods

### *Experimental protocol*

Twenty-four Japanese white rabbits weighing between 2.4 ve 3.5 kg were initially anaesthetised with intramuscular ketamine hydrochloride (50 mg/kg) and xylazine (5mg/kg), followed by a half-dose maintenance as required during the procedure. The animals maintained spontaneous breathing. To monitor proximal and distal aorta blood pressure, two catheters was placed into the aorta and femoral arteries. The ear vein was also cannulated for the administration of fluids. The base deficit was corrected with administration of sodium bicarbonate. A thermister-mounted probe was inserted into the rectum to monitor the core temperature. After sterilization, the abdomen was entered through a median laparotomy and the abdominal aorta was exposed just inferior to the left renal artery and down to the aortic bifurcation. The aorta was encircled with a silk ligature distal to the renal artery and proximal to the bifurcation to facilitate secure occlusion. The posterior mesenteric artery was also encircled with a silk ligature. A heating pad was employed to keep the core temperature at 38°C to 39°C during the aortic occlusion period. Each rabbit was anticoagulated

with 200 units of heparin before aortic cross-clamping. The aorta was occluded distal to the left renal artery with a pediatric vascular clamp (De Bakey-Hess, A-237, Aygün) and proximal to the bifurcation with a similar clamp. The posterior mesenteric artery was also occluded with a clamp. After releasing aortic occlusion, the abdomen was closed in to layers and the subjects were allowed to recover. Forty-eight hours postoperatively, the motor function of the lower limbs was evaluated in each subject according to the Tarlov Scale by an independent observer who was unaware of the treatment modality of each subject. The subjects were then sacrificed by intracardiac formalin (10%). The lumbar spinal cord was harvested and fixed in a 10% phosphate-buffered formalin solution. Sections were obtained from lower lumbar regions, stained with hematoxylin and eosine, and were studied for ischemic injury using standard light microscopy.

The subjects were randomly divided into four groups with 6 subjects in each group (Table 1). Groups I (control, n=6) was anaesthetised and subjected to laparotomy without aortic occlusion. In group II (ischemia), subjects were subjected to 30-minute of crossclamping. In group III (preconditioning group), subjects were subjected to 5-minute of crossclamping. In ischemic preconditioning subjects (groups IV), a 5-minute crossclamping with 10 minutes of reperfusion followed by another 30-minute crossclamping was performed (13,15-16).

Spastic paraplegia did not produced with the 5-minute occlusion time in all rabbits (group III). Therefore, this occlusion time was selected to test the protective effects of ischemic preconditioning (group IV).

**Table 1.** Tarlov Scale

Tarlov Scale	Description
0	No voluntary movement, spastic paraplegia
1	Perceptible movement of the hind limbs
2	Good movement but unable to stand
3	Able to stand and walk
4	Complete recovery

### Neurologic examination

Neurologic assessment was performed at 48 hours after the procedure. Subjects were classified according to the method of Tarlov scale (Table 1).

### Histopathologic examination

Transverse sections were cut to a thickness of 4µm at the level of L6. The sections were stained with hematoxylin-eosin and examined by light microscopy. Morphologic evaluation of spinal cord samples of all groups were performed by examiners not informed about group specificities.

### Statistical Analysis

Statistical analysis of the neurologic scores was performed with the nonparametric Mann-Whitney U test. All other data are presented as means  $\pm$  SD. Intergroup and intragroup mean values were compared by repeated measures ANOVA and post Hoc Tukey. Differences were considered statistically significant at the  $P < 0.05$  level.

The experimental protocol was approved by the Dicle university institutional Animal Care and Use committee. All animals received humane care in compliance with the European Convention on Animal Care.

## Results

There was no statistical difference in the heart rate, proximal and distal arterial pressure values among groups (Table 2). All subjects in which an aortic clamp had been applied (groups II-IV) demonstrated significant differences between baseline and postocclusion pressures both in the proximal aorta and in the femoral artery ( $p < 0,05$  and  $p < 0,001$ , respectively).

All subjects tolerated the procedure well and were in stable condition at the time of evaluation of lower limb motor functions. The ratio of the specific grades of each group is shown in Table 3. The rabbits in control group (group I) showed no neurologic deficit. Paraplegia (scale 0) developed in all rabbits in the ischemia group (group II). Of the group III (preconditioning group), 5 animals were Tarlov scale 4, whereas one animal was Tarlov scale 3. Ischemic preconditioning subjects (groups IV) resulted in significant reduction of motor disfunction ( $p = 0,002$ ) (Table 3).

Results of the blinded histologic study fully correlated with neurologic findings. There were no histopathologic abnormalities in hematoxylin-eosin-stained section in sham-operated rabbits. The cords of subjects with Tarlov's scale 3 and 4 showed only minimal cellular infiltrates in the gray matter, and there was significant preservation of nerve cells (Fig 1a). In histologic evaluation of the spinal cord sample of the subjects paraplegia, severe neuronal injury was the prominent finding. Necrotic neurons either appeared shrunken with a condensed perikaryon or displayed eosinophilic cytoplasm and pyknotic nuclei (Fig 1b).

## Discussion

Postoperative paraplegia is caused by the ischemic injury of the spinal cord and is related to thoracic aortic crossclamping during operations, because critical intercostal arteries supplying the anterior spinal artery may be occluded. Technical efforts in order to decrease the incidence of spinal cord injury are usually directed towards protection of the cord during the ischemic interval and prompt

**Table 2.** Haemodynamic parameters\*

	Baseline				Occlusion				Reperfusion			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Heart rate (bpm)	184 $\pm$ 6	187 $\pm$ 4	184 $\pm$ 5	186 $\pm$ 6	194 $\pm$ 7	191 $\pm$ 6	193 $\pm$ 4	193 $\pm$ 5	191 $\pm$ 6	193 $\pm$ 5	192 $\pm$ 5	193 $\pm$ 7
Mean Proximal pressure (mmHg)	54,7 $\pm$ 5	55,3 $\pm$ 4	53,6 $\pm$ 6	54,0 $\pm$ 6	54,2 $\pm$ 5	68,7 $\pm$ 6	66,5 $\pm$ 5	68,2 $\pm$ 7	53,5 $\pm$ 4	54,8 $\pm$ 5	55,2 $\pm$ 4	55,0 $\pm$ 6
Mean distal pressure (mmHg)	53,8 $\pm$ 5	53,0 $\pm$ 4	53,8 $\pm$ 4	54,5 $\pm$ 6	54,3 $\pm$ 4	14,2 $\pm$ 4	14,0 $\pm$ 5	13,6 $\pm$ 6	52,8 $\pm$ 5	53,5 $\pm$ 6	52,7 $\pm$ 4	54,2 $\pm$ 6

\*There was no statistical difference in the heart rate, proximal and distal arterial pressure values among groups. All rabbits in which an aortic clamp had been applied (groups II-IV) demonstrated significant differences between baseline and postocclusion pressures both in the proximal aorta and in the femoral artery ( $p < 0,05$  and  $p < 0,001$ , respectively).

**Table 3.** Tarlov score at 48 hours of reperfusion\*

Tarlov score	Group I (Control) (n=6)	Group II (Ischemia) (n=6)	Group III (Preconditioning) (n=6)	Group IV (Ischemic preconditioning) (n=6)
0	0	6	0	0
1	0	0	0	2
2	0	0	0	2
3	0	0	1	1
4	6	0	5	1
Mean±SD	4	0	3,8±0,4	2,2±1,1

\*Mean Tarlov Scales of rabbits showed a significant difference between ischemia (group II) and ischemic preconditioning (group IV) groups. Ischemic preconditioning rabbits (groups IV) resulted in significant reduction of motor dysfunction (P = 0,002)

**Fig 1a.** Neuronal cells are well preserved. **b.** Necrotic neurons exhibiting shrinkage, anuclear, darkly, triangular-shaped cell are seen (hematoxylin-eosin stain; original magnification x100)

reestablishment of blood flow to the spinal cord as a part of the repair (16).

Since the advent of ischemic preconditioning in myocardium, incremental attention has been paid to ischemic preconditioning in the central nervous system and spinal cord (13-14,17-18). In models of cerebral ischemia, the preconditioning stimulus is typically a period of non-lethal, brief ischemia secondary to vascular occlusion, and this protective state induced many hours to days later is referred to as ischemic tolerance (12). Fan et al. (15) showed that 5 minutes of ischemic preconditioning may increase the regional spinal cord blood flow, enhance the tolerance of the spinal cord to irreversible ischemia, and protect the neurofunction of the spinal cord in rabbits.

The protective mechanisms underlying ischemic preconditioning may involve adenosine,

enhanced mitochondrial sequestration of Ca<sup>2+</sup>, more rapid recovery of protein synthesis, or induction of heat-shock proteins after preconditioning (19-22). In addition, differences in the activation of microglia (cytotoxic) and astroglia (neurotrophic) may play a role in ischemic preconditioning (23).

Despite the fact that several studies have shown ischemic preconditioning to have a protective effect on the spinal cord there is still some doubt as to ischemic preconditioning duration. Zyara et al. (14) reported that protective effect of ischemic preconditioning occurred after 3 min of ischemia and 30 min of reperfusion interval. Ueno et al. (24) showed significantly increased postischemic lumbar cord blood flow and contributed to lower incidence of spastic paraplegia. Abraham et al. (17) reported a protective effect of ischemic preconditioning after

2 and 5 min of ischemia and 48 h of reperfusion interval. Comparisons between the results of different studies are difficult as owing to the differences in species, experimental protocols and outcome measures. However one important thing is that, ischemia must be present at least 5 min to elicit the phenomenon (15). We applied a short period of ischemic preconditioning (5 min) in order to be similar to application in experimental studies. In our study, preconditioning protection subjects (group IV) resulted in a reduced incidence of severe motor dysfunction. In contrast, all subjects in group II (ischemia group) were paraplegic with no sensory or motor reflexes. This improvement of neurologic function rates and the histopathological findings reveal the protective effect of preconditioning on spinal tissue against ischemia-reperfusion injury.

Finally these findings suggests that preconditioning reduces ischemia-reperfusion damage in the spinal cord and provides a better neurologic outcome. Conclusions inferred in experimental models such as the present report may not be readily applicable to humans. Thus, further study is needed to define the prerequisite preconditioning and reperfusion times for necessary for maximal benefits.

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