



# Short-interval postconditioning protects the bowel against ischaemia–reperfusion injury in rats

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## Abstract

**Objective:** Acute mesenteric ischaemia leads to intestinal damage. Restoration of blood flow results in further damage to tissue, which is called reperfusion injury. This study aimed to investigate the protective effects of short-interval postconditioning and to determine the optimal interval for reperfusion in an experimental rat model of intestinal ischaemia.

**Methods:** Forty adult male Wistar rats were grouped as follows: sham (Sh), ischaemia + reperfusion (IR), ischaemia + postconditioning for 5 seconds (PC5), ischaemia + postconditioning for 10 seconds (PC10), and ischaemia + postconditioning for 20 seconds (PC20). For postconditioning, 10 cycles of reperfusion (5, 10, or 20 seconds) interspersed by 10 cycles of 10 seconds of ischaemia were performed. Blood glutathione reductase (GR) and glutathione peroxidase (GPx) levels were measured. Intestinal tissue damage was assessed histopathologically.

**Results:** GR levels were significantly higher in the PC5 group than in the IR group ( $37.7 \pm 9.0$  vs.  $18.5 \pm 2.0$  min/g Hb). GPx levels were significantly higher in the PC10 group than in the IR group ( $43.2 \pm 9.2$  vs.  $15.9 \pm 4.6$  U/g Hb). The histopathological score was significantly lower in the PC5 group ( $1.1 \pm 0.1$ ) than in the IR group ( $2.1 \pm 0.2$ ).

**Conclusion:** Short-interval postconditioning reduces reperfusion injury in the ischaemic bowel and the optimal interval for reperfusion is 5 seconds. The long-term effects of short-interval postconditioning and the optimal reperfusion interval in intestinal ischaemia–reperfusion in rats need to be investigated.

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## Keywords

Intestine, ischaemia, oxidative stress, postconditioning, reperfusion injury

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## Introduction

Acute mesenteric ischaemia occurs as a result of decreased blood flow to the intestine because of several reasons, such as systemic hypotension, major cardiovascular surgery, trauma, and midgut volvulus or intussusception in infants. This can lead to intestinal ischaemia and ischaemic injury.<sup>1</sup> Moreover, some bowel surgeries, such as intestinal resection or transplantation, are performed through occlusion of mesenteric arteries for preventing bleeding.<sup>2</sup> A decrease in blood flow to the bowel leads to hypoxia, and this causes elevated levels of oxygen-derived free radicals. After blood flow is restored in this ischaemic tissue, more oxygen-derived free radicals are released. These oxygen-derived free radicals cannot be exterminated by enzymatic antioxidant defense systems. This phenomenon is called reperfusion injury and this leads to further tissue injury.

The bowel is the most sensitive organ to ischaemia–reperfusion injury among the abdominal organs.<sup>3</sup> Intestinal cells can be easily injured during the ischaemia–reperfusion period and mucosal injury leads to an increase in intestinal permeability and in systemic bacterial translocation.<sup>4,5</sup>

Several methods have been used to decrease tissue ischaemia–reperfusion injury.

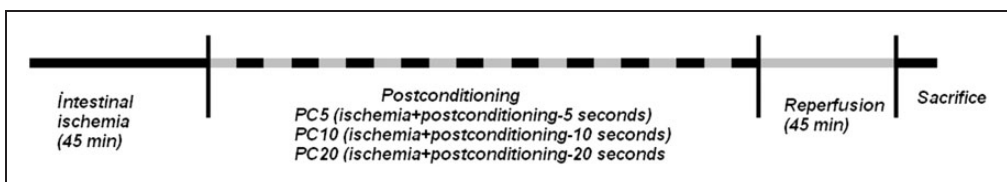
Zhao et al.<sup>6</sup> were the first authors to report postconditioning. This technique consists of performing a few short periods of intermittent reperfusion applied just after an ischaemic episode and before reperfusion occurs. This method has been used in some clinics and animals models to decrease reperfusion injury.<sup>7–9</sup>

This study aimed to assess the preventive effects of short-interval PC (SIPC) and to determine the optimal intervals of reperfusion for PC in an experimental rat model of intestinal ischaemia (Figure 1).

## Material and methods

This study was performed in the Experimental Laboratory of Adnan Menderes University and was approved by the Local Ethics Committee. Forty adult male Wistar rats were used. The rats were randomly divided into five groups as follows: sham-operated (Sh,  $n = 8$ ); ischaemia + reperfusion (IR,  $n = 8$ ), ischaemia + postconditioning for 5 seconds (PC5,  $n = 8$ ), ischaemia + postconditioning for 10 seconds (PC10,  $n = 8$ ), and ischaemia + postconditioning for 20 seconds (PC20,  $n = 8$ ).

Briefly, anaesthesia was performed using ketamine (50 mg/kg) and xylazine (3 mg/kg) injection intramuscularly. Surgery was performed via median laparotomy.



**Figure 1.** Experimental protocol and time line of short-interval postconditioning.

In the IR, PC5, PC10, and PC20 groups, the superior mesenteric artery was clamped together with its collaterals using an atraumatic vessel clamp for 45 minutes to induce ischaemia. No arterial pulsation was observed in the intestinal segments during the ischaemic period.

After the ischaemic period, in the IR group, the vessel clamp was opened for 45 minutes. In the PC5 group, the vessel clamp was opened for 5 seconds, and immediately after this time, the artery was occluded by vessel clamp for 10 seconds. In the PC10 group, the vessel clamp was opened for 10 seconds, and immediately after this time, the artery was occluded by vessel clamp for 10 seconds. In the PC20 group, the vessel clamp was opened for 20 seconds, and immediately after this time, the artery was occluded by vessel clamp for 10 seconds. In all of the PC groups, the interval procedure was repeated 10 times. When the procedure was finished, 45 minutes of reperfusion was performed in all of the PC groups and in the IR group. In the Sh group, no intervention was performed.

Intracardiac blood samples were obtained from all of the rats to determine blood glutathione reductase (GR) and glutathione peroxidase (GPx) levels immediately after 45 minutes of reperfusion. All of the rats were sacrificed after blood and intestinal samples were obtained for histopathological and biochemistrical evaluation.

### Histopathological analysis

For histopathological evaluation, tissue damage in the experimental groups was evaluated using a six-level scoring system.<sup>10</sup> This scoring system was as follows: degree 0, normal; degree 1, mucosal injury and no necrosis; degree 2, necrosis of villi and crypts are normal; degree 3, necrosis of villi and crypts; degree 4, deeper muscular or full-thickness mucosal necrosis; degree 5, full-thickness necrosis.

### Statistical analysis

The Mann–Whitney U test was used for statistical analyses.

### Results

The biochemical parameters and histopathological scores in the study groups are shown in Table 1. The mean GR level in the PC5 group was similar to that in the Sh group. The mean GR level was significantly higher in the PC5 group than in the IR group ( $P=0.04$ ). The mean GPx level in the PC10 group was significantly higher than that in the IR group ( $P=0.02$ ). Histopathological evaluation showed that the mean histopathological score in the PC5 group was lower than that in the IR group ( $P=0.01$ ).

### Discussion

Oxygen-derived free radicals cause tissue damage and their peak production occurs in the first few minutes after reperfusion. The peak production of oxygen-derived free radicals during this initial phase of reperfusion is the major factor of

**Table 1.** Blood glutathione reductase and glutathione peroxidase levels, as well as histopathological scores in the study groups.

Groups	GR levels (min/g Hb)	GPx levels (U/g Hb)	Histopathological damage score
Sh	40.5 ± 13.4	57.8 ± 23.5	0.0 ± 0.0
IR	18.5 ± 2.0	15.9 ± 4.6	2.1 ± 0.2
PC5	37.7 ± 9.0*	40.1 ± 19.4	1.1 ± 0.1*
PC10	25.5 ± 3.2	43.2 ± 9.2*	1.3 ± 0.2
PC20	20.9 ± 4.8	19.9 ± 5.6	1.6 ± 0.3

\* $P < 0.05$  compared with the IR group.

GR, glutathione reductase; GPx, glutathione peroxidase; Sh, sham-operated; IR, ischaemia + reperfusion; PC5, ischaemia + postconditioning for 5 seconds; PC10, ischaemia + postconditioning for 10 seconds; PC20, ischaemia + postconditioning for 20 seconds.

reperfusion injury.<sup>11</sup> Some methods have been used to minimize reperfusion damage. One of these methods is PC, which was first reported by Zhao et al.<sup>6</sup> in an experimental cardiac ischaemic model. The model by Zhao et al.<sup>6</sup> includes several short reperfusion cycles immediately after ischaemia and before reperfusion. With this method, preventing the sudden increase in production of free radicals in the ischaemic area during reperfusion is possible.

Reperfusion injury has two phases. The first phase is the early phase and the second is the late phase. The early phase is shortly after reperfusion and continues for a few hours. The late phase continues longer and is associated with nonreversible tissue injury. Ozkisacik et al.<sup>12,13</sup> showed that SIPC prevented testicular damage in the short and long term in a rat model of testicular torsion. They performed 10 cycles of reperfusion (5 seconds in the PC5 group, 10 seconds in the PC10 group, and 20 seconds in the PC20 group) interspersed by 10 cycles of ischaemia (10 seconds in all groups) for SIPC. They found the optimal reperfusion interval for PC was 5 seconds to prevent ischaemia–reperfusion injury in their experimental model. They reported that SIPC led to a decrease and then an immediate increase in reactive oxygen species during the early phase and tissue injury. They also suggested that PC reduced late phase tissue injury via reduced neutrophil and macrophage deposition, and thereby reduced reperfusion injury. In our study, we found high GR and GPx levels in the early phase of the reperfusion period in blood samples immediately after ischaemia. Therefore, this appears to be a preventive effect in the intestinal ischaemia–reperfusion model.

Some studies have evaluated the preventive effect of PC in bowel ischaemia–reperfusion injury. Rosero et al.<sup>14</sup> investigated the optimal PC algorithm in a rat model of intestinal ischaemia.

They performed PC with five cycles of 10 seconds, 30 seconds, and 1 minute for each cycle after a 60-minute ischaemic period. They found that ischaemic PC was not able to minimize or prevent intestinal tissue injury in rats undergoing ischaemia and the reperfusion process. Nakamura et al.<sup>15</sup> also reported that short cycles of PC were not effective in preventing intestinal tissue injury in rats. In their study, they performed 5 cycles of 30 seconds of reperfusion interspersed by 5 cycles of 30 seconds of ischaemia. Based on these two studies, we considered that if the PC interval time was shorter than that in these studies it could protect the bowel against reperfusion injury. In the present study, the PC intervals (5, 10, and 20 seconds) were shorter than those in the studies by Nakamura et al.<sup>15</sup> and Rosero et al.<sup>14</sup> and SIPC was able to protect the bowel against reperfusion injury. In this study, we obtained beneficial results in the PC5 group, in which 10 cycles of 5 seconds of reperfusion interspersed by 10 cycles of 10 seconds of ischaemia was performed for PC.

GPx catalyzes the peroxidation of hydrogen peroxide in the presence of reduced glutathione to form water and oxidized glutathione. Several studies have demonstrated the protective effect of GPx and GR in ischaemia–reperfusion injury in various tissues.<sup>16</sup> In our intestinal ischaemia–reperfusion model, the mean level of GR in the PC5 group was significantly higher than that in the IR group. Moreover, comparison of GPx levels among the groups showed that the mean GPx level was significantly higher in the PC10 group than in the IR group. Therefore, we concluded that tissue damage was least with the reperfusion intervals of 5 and 10 seconds for PC.

In the present study, intestinal tissue damage was also assessed histopathologically. We found that among the study groups, intestinal damage was lowest in the PC5 group. Therefore, the tissue-protective effect

of PC was reduced with increasing reperfusion intervals of PC.

The present study shows that SIPC can minimize reperfusion injury in the intestinal ischaemia–reperfusion model in rats. This technique is easily applicable. The optimal reperfusion interval was 5 seconds because higher blood GR levels and the lowest histopathological grade were observed with this reperfusion interval. We observed that as the reperfusion interval became prolonged, the tissue-protective effect of PC was reduced in the present experimental model, which is similar to our previous testicular torsion model in rats.<sup>12</sup> Therefore, intestinal damage could be decreased by SIPC during the first phase of reperfusion. Further studies are required to investigate the long-term effects of SIPC and the optimal reperfusion interval in intestinal ischaemia–reperfusion in rats.

### Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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