Effect of Ischemic Preconditioning on Injuries Caused by Ischemia and Reperfusion in Rat Intestine


ABSTRACT
To study whether ischemic preconditioning (IPC) attenuated intestinal dysfunction caused by ischemia (I) and reperfusion (R), rats were underwent 60 minutes of I which was produced by occlusion of the superior mesenteric artery, and/or 120 minutes R. The IPC group had the I procedure previously stimulated for 5 minutes and the R for 10 minutes. IPC and sham groups were injected with saline solution (SS) via the femoral vein 5 minutes before the I and R, and for R. After I or I/R, 2-cm jejunal segments were mounted in an organ bath to study neurogenic contractions stimulated by electrical pulses or KCl using a digital recording system. Thin jejunal slices were stained with hematoxylin and eosin for optical microscopy. Compared with the sham group, jejunal contractions were similar in the IPC + I and the IPC + I/R groups, but reduced in the I + SS and the I/R + SS groups. The jejunal enteric nerves were damaged in the I + SS and the I/R + SS groups, but not in the IPC groups. These results suggested that ischemic preconditioning attenuated intestinal dysfunction caused by I and I/R.

INTESTINAL ISCHEMIA (I) and reperfusion (R) injuries occur in a variety of surgical practices, such as transplantation procedures and critical illnesses, such as neonatal necrotizing enterocolitis. Among the internal organs the intestine is probably the most sensitive to the I/R syndrome. I and R injuries can lead to hypovolemia, hypotension, hypoxia, and sepsis, dramatically compromising intestinal motor and secretory functions due to cellular lesions caused by deprivation of oxygen and nutrients. In addition, vessels that suffer R after I can also suffer cellular lesions and death mainly due to lipid peroxidation of cell membranes caused by accumulation of free oxygen radicals and other cytotoxic substances. In response to I, cells undergo changes in enzyme activities, mitochondrial functions, cytoskeletal structures, membrane transport, and antioxidant defenses, which collectively predispose to reoxygenation injury. The morphological and functional injuries after I and R remain a major challenge associated with high rates of morbidity and mortality both in surgical and trauma patients.

The motor functions of the mammalian intestines are regulated by several excitatory and inhibitory transmitters, which are released from enteric nerves, including acetylcholine, 5'-adenosine triphosphate (ATP), nitric oxide, neuropeptides, and other substances. The actions of these transmitters are highly dependent on the integrity of the enteric nerves. However, intestinal motor activity is severely compromised in I/R mainly due to the loss of structural and functional integrity of the enteric nerves.

Recent studies have suggested ischemic preconditioning (IPC) to be a potential therapeutic strategy to improve the tolerance of the small intestine to ischemic and preservation insults. IPC, which was first described by Murry et al., refers to a phenomenon in which a tissue is rendered resistant to the deleterious effects of prolonged I and R by prior brief periods of vascular
occlusion.\textsuperscript{13} However, both in vivo and in vitro models have shown the cycle of preconditioning to be critical to inducte tolerance to ischemia. Hypoxic periods shorter than 5 minutes or exceeding 15 minutes failed to induce protection.\textsuperscript{21} Similarly, R periods shorter than 5 or exceeding 15 minutes had no protective effect.\textsuperscript{21} Although most reports in the literature have used 10 minutes of IPC in rat small bowel I/R injury, the ideal IPC protocol, including the duration of I, is not yet established.\textsuperscript{22}

Wang et al demonstrated that the protective effects of IPC on small intestinal grafts correlated with inhibition of epithelial cell apoptosis.\textsuperscript{14} Other studies have shown several mediators to play crucial roles in this protective phenomenon, including adenosine,\textsuperscript{15} nitric oxide (NO),\textsuperscript{16} oxidative stress,\textsuperscript{17} heme-oxygenase-1\textsuperscript{18} and protein kinase C (PKC).\textsuperscript{19} Since the molecular mechanisms and ideal protocol involved in IPC are still not fully known, we sought to study the effects of IPC on the motility and histology of rat jejunal segments undergoing intestinal I and R in rats.

MATERIALS AND METHODS
Male Wistar EPM-1 Rats (270–300 g) were anesthetized with ketamine (60 mg · kg\textsuperscript{-1}) and xylazine (40 mg · kg\textsuperscript{-1}) intravenously prior to occlusion of the superior mesenteric artery using a metallic clip for 60 minutes (I) followed by removal of the clip with recirculation (R) for 120 minutes. Twelve rats underwent only I (I group) and 12, I and R (I/R group) in addition to a sham group (n = 6).

Among the I group, six rats were treated only with saline solution 0.9% (SS) (I + SS) via the femoral vein and six...
underwent IPC (IPC + I) for 5 minutes before I. In the I/R group, six rats were treated with SS and six underwent the IPC (IPC + I/R) 10 minutes before R. Both IPC and sham groups were injected with SS via the femoral vein 5 minutes before the I and 10 minutes before the R.

After I or I/R, the rats were sacrificed to retrieve jejunal segments (2 cm) that were isolated, washed, cleared of surrounding tissues, and mounted under 1 g tension at 37°C in an organ bath containing 10 mL of arterial nutrient solution of composition (in mmol/L): NaCl 138, KCl 5.7, CaCl2 1.8, NaH2PO4 0.36, NaHCO3 15, and dextrose 5.5 (pH 7.4). Using a digital recording system we studied neurogenic contractions induced either by electrical field stimulation (EFS) or by the depolarizing agent KCl (70 mmol/L).6–8 EFS (5 and 30 Hz, 1-ms duration, 60 V) was performed using platinum electrodes connected to an S88 electrical stimulator (Grass, USA).6–8

Responses to EFS and KCl were recorded by force-displacement transducers connected via a bridge amplifier to an analog/digital recording system (AD Instruments, USA). Data on contractile responses were subjected to statistical analysis using one-way analysis of variance and Student t test.6-8 We also performed histological analyses using optical microscopy of jejunal pieces embedded in paraffin, cut into thin slices, and stained with hematoxylin and eosin.

### RESULTS

Figure 1 shows that EFS (5 and 30 Hz) and KCl (70 mmol/L) produced contractile responses in all jejunal segments: sham, I + SS, IPC + I, I/R + SS, IPC + I/R. However, the amplitude of these contractions were similar in IPC + I and IPC + I/R groups, but reduced in I + SS and I/R + SS groups compared with the sham group (Fig 1 and Table 1).

Histological analysis showed a loss of structural integrity of enteric nerves in the jejunal segments of I/R + SS group (Fig 2B), but in I/R + HEP (Fig 2C).

### DISCUSSION

Our results showed that the amount of and the motility stimulated by transmitters released by enteric nerves were significantly reduced in jejunal segments from rats undergoing intestinal I and R. The deleterious effects of R on the intestine exacerbated the I injury.15 Molecular mechanisms involved in the I/R injury are poorly understood, but involve formation of free oxygen radicals (ROS) with consequent oxidative stress, alterations of calcium flux, and activation of phospholipase A2. The stressed or injured tissues release endogenous adenosine, which blocks potentially destructive inflammatory cascades, decreasing activation of platelets, leukocytes, endothelial cells, and responses that are mediated mainly by A2A and A2B purinoceptors.9-12 However, these dysfunctions were reduced or absent among rats subjected to IPC, suggesting that the intestine becomes resistant to the deleterious effects of prolonged I and R following prior exposure to brief periods of vascular occlusion.

The intracellular signaling pathway for IPC in intestines undergoing I/R injury has not been completely clarified. Probably more than one pathway acts in sequence or parallel.23,24 The IPC phenomenon, can be divided into trigger and mediator phases:23 the first triggering phase involves the release of adenosine, bra-

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### Table 1. Values of Amplitude of Neurogenic Contractions (Expressed in Grams of Tension) Induced by Electrical Field Stimulation (5 and 30 Hz) or KCl (70 mmol/L) in Jejune of Rats Treated with IPC or Saline Solution and Submitted to Intestinal I or I/R

<table>
<thead>
<tr>
<th>Groups</th>
<th>5 Hz</th>
<th>30 Hz</th>
<th>KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>1.43 ± 0.12</td>
<td>2.15 ± 0.19</td>
<td>2.25 ± 0.18</td>
</tr>
<tr>
<td>I + SS</td>
<td>0.18 ± 0.04†</td>
<td>0.28 ± 0.05‡</td>
<td>0.41 ± 0.10*</td>
</tr>
<tr>
<td>IPC + I</td>
<td>1.01 ± 0.12†</td>
<td>1.20 ± 0.19†</td>
<td>1.73 ± 0.25†</td>
</tr>
<tr>
<td>I/R + SS</td>
<td>0.18 ± 0.06</td>
<td>0.45 ± 0.10</td>
<td>0.61 ± 0.17</td>
</tr>
<tr>
<td>IPC + I/R</td>
<td>1.22 ± 0.15‡</td>
<td>1.27 ± 0.19£</td>
<td>1.66 ± 0.23£</td>
</tr>
</tbody>
</table>

Data corresponding to means ± standard errors of the means (n = 6). I, ischemia; R, reperfusion; IPC, ischemic preconditioning.

*Statistically different from sham (P < .05).
†Statistically different of I/R (P < .05).
‡Statistically different of I + SS (P < .05).
§Statistically different of IPC (P < .05).

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**Fig 2.** Histological aspects of jejune of sham rat (A) (without ischemia and/or reperfusion) and treated with saline solution (B) or ischemic preconditioning after ischemia and reperfusion (C) and submitted to intestinal ischemia and/or reperfusion. The images show the longitudinal muscle (ME), circular muscle (MI), and enteric nerves (black arrows). (hematoxylin and eosin method, 400×).
dykinin, opioids, and even catecholamines, as well as free radicals and other triggers. These pathways eventually activate PKC directly or indirectly, leading to downstream phosphorylation of endothelial nitric oxide synthase and NO formation, which in turn activates soluble guanylyl cyclase (GC) and protein kinase G (PKG). The latter phase initiates opening of mitochondrial $K_{ATP}$ channels and generation of ROS that targets PKC. Once this pathway is activated, tissues can be protected for a long time, that is, the heart enters a protected phenotype that persists for an hour or more even after the triggering agonists have been washed away.

Some events in the mediator phase are less well defined. PKC and phospholipase C seem to activate phosphatidylinositol 3-kinase, AKT, nitric oxide synthase, GC and PKG, leading to inhibition of the mitochondrial permeability transition pore (MPTP) that controls the release of calcium and proapoptotic factors into the cytoplasm, thereby improving cytoprotection. In addition, downstream PKC activation of mitogen-activated protein kinase alters, expression and antiapoptotic protein genes like Bcl-2.

Some authors have demonstrated that PKC modifies an $A_{2B}$ purinoceptor: endogenous ADO binds to it early in the R period. Many downstream signaling events of $A_{2B}$ purinoceptors recapitulate those in the trigger pathway. This signaling is thought to ultimately prevent opening of the MPTP. If the latter are allowed to form, mitochondrial matrix abolishes the critical electrochemical equilibrium between the cytoplasm and mitochondria, thereby improving cytoprotection. In addition, downstream PKC activation of mitogen-activated protein kinase alters, expression and antiapoptotic protein genes like Bcl-2.

REFERENCES


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