Comparative Medicine
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Vol 57, No 2
April 2007
Pages 200–205

Brief Small Intestinal Ischemia Lessens Renal Ischemia-reperfusion Injury in Rats

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Ischemic preconditioning (IPC) not only reduces local tissue injury caused by subsequent ischemia-reperfusion (IR) but may also have a beneficial effect on IR injury of tissues remote from those undergoing preconditioning. In this study, we investigated the effect of small intestinal IPC on renal IR injury in rats. Renal IR injury was induced by a 45-min renal artery occlusion and reperfusion for 2 or 24 h in rats with a previous contralateral nephrectomy, and ischemic preconditioning was induced by 3 cycles of 8-min ischemia and 5-min reperfusion of the small intestine. We then measured the concentrations of plasma creatinine (Cr) and blood urine nitrogen (BUN) and the level of malondialdehyde (MDA) and activities of superoxide dismutase (SOD) and catalase (CAT) in the renal cortex. Renal histopathology also was evaluated. Pretreatment with intestinal ischemic preconditioning significantly alleviated renal IR injury, as shown by decreases in the levels of Cr, BUN, and MDA, decreased renal morphologic change, and improved preservation of SOD and CAT activities. These results suggest that remote ischemic preconditioning of the small intestine protects against renal IR injury by inhibition of lipid peroxidation and preservation of antioxidant enzyme activities.

Abbreviations: BUN, blood urea nitrogen; CAT, catalase; Cr, creatinine; IPC, ischemic preconditioning; IR, ischemia-reperfusion; MDA, malondialdehyde; NBT, nitroblue tetrazolium; rIPC, remote preconditioning; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid

Ischemic preconditioning (IPC) is a phenomenon whereby a brief episode of nonlethal ischemia produces protection against a subsequent detrimental ischemia-reperfusion (IR) insult. IPC was described initially in heart[21] and subsequently has been demonstrated in brain,[14] liver,[26] skeletal muscle,[5] lung,[18] and small intestine.[30] In addition, it recently was reported that an ischemic episode in 1 organ can not only augment ischemic tolerance within the same organ but also can have a remote effect on another organ.[10] This phenomenon of cross tolerance has been termed remote preconditioning (rIPC).

Despite important advances in critical care medicine, the mortality from acute renal failure attributed to IR injury remains high.[31] In addition, despite advanced improvements in organ procurement and formulation of perfusates, renal damage resulting from IR is unavoidable in renal transplantation, and IR remains one of the leading causes of the early loss of transplanted organs.[3,11] Therefore, more effective ways to protect against renal IR injury are needed urgently. There is substantial evidence to suggest that IPC is perhaps the most powerful endogenous protective mechanism to protect against IR injury in cardiac and noncardiac tissues.[35] Although renal IPC is reported to exist in animals and humans,[13,16,27,34] little or no attention has been paid to renal protection by remote organ IPC. Ates and coworkers[1] have reported that 10-min hepatic ischemia with 10-min reperfusion affords functional and morphologic protection in rat kidney that undergoes a subsequent 45-min ischemic insult.[1]

It increasingly is recognized that intestinal IR is a primary effector of multisystem organ failure (MSOF). Interestingly, IPC of the small intestine not only elicits a local protective effect but also can protect against heart IR injury.[10,38] However, the question of whether small intestinal IPC exerts protective effects on remote noncardiac tissues remains unclear. Therefore, in the present study, we examined the remote protective effect of small intestinal IPC on renal IR injury in rats.

Received: 12 Jul 2006. Revision requested: 13 Oct 2006. Accepted: 1 Nov 2006.
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Materials and Methods

Animals. Specific-pathogen-free male Wistar rats (n = 64) weighing 250 to 300 g were used and housed in an air-conditioned room with 12:12-h light:dark cycles and constant temperature (22 ± 2 °C) and relative humidity (65% to 70%) at the Laboratory Animal Center (Xiang-Ya School of Medicine, Changsha, China). The rats had free access to a standard diet and tap water until use. All animals received humane care in compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals published by the National Institutes of Health (Public Law 99-158, revised 1986, reprinted 2000),[37] and this study was approved by the Central South University Veterinary Medicine Animal Care and Use Committee. Rats were monitored and maintained to be free of the following pathogens: *Salmonella* sp., *Listeria monocytogenes*, *Yersinia pseudotuberculosis*, *Y. enterocolitica*, pathogenic dermal fungi, *Pasteurella multocida*, *P. pneumotropica*, *Bordetella bronchiseptica*, *Mycoplasma pulmonis*, *Corynebacterium kutscheri*, *Bacillus piliformis*, epidemic hemorrhagic fever virus, Sendai virus, rat parvovirus, sialodacryoadenitis virus, pneumonia virus of mice, murine adenovirus, *Toxoplasma gondii*, *Taenia* sp., *Hymenolepis* sp., *Syphacia* sp., *Aspiculuris tetraptera*, *Trichosomoides crassicauda*, and ectoparasites.

Surgical procedure and experimental protocols. The rats were anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally and supplemented as needed to maintain a surgical anesthetic

level). Right nephrectomy was performed. Briefly, the abdominal region was shaved, and each animal was placed on a heating pad to maintain rectal temperature at 37 to 38 °C throughout surgery. The abdominal area then was scrubbed with povidone iodine followed by wiping of the surgical site with isopropyl alcohol. A midline incision was performed, and the intestines were displaced upward to expose the right kidney. After blunt dissection of the right kidney from its bed, the right renal artery and vein were identified and ligated with silk suture, and the right kidney was harvested. The abdomen was closed in 2 layers. Buprenorphine (0.05 mg/kg subcutaneously) was administered immediately after surgery, and then ibuprofen (0.15 mg/ml) was administered in the drinking water for 48 h to minimize postoperative pain. The rats were allowed to recover for 2 wk before they underwent IR injury.

On the 15th day after right nephrectomy, a second laparotomy was performed under the same anesthesia condition, the anterior mesenteric and renal arteries were dissected free, and a suture was placed around the arteries to facilitate occlusion with a nontraumatic microvascular clamp. Occlusion of the anterior mesenteric and renal arteries caused ischemia. Reperfusion was achieved by releasing the clamp. The abdomen was closed, and fluid losses were replaced by intraperitoneal administration of 5 ml of warm (37 °C) isotonic saline after surgery. All animals survived until termination of the study.

Rats were allocated randomly into 4 groups. In the IR groups, the animals underwent 45 min of renal artery occlusion followed by reperfusion for 2 (n = 16) or 24 (n = 16) h without occlusion of the anterior mesenteric artery. In the intestinal IPC+IR group (n = 16), rats underwent 3 cycles of 8-min anterior mesenteric artery occlusion followed by 5-min reperfusion before renal artery occlusion. The sham group (n = 16) underwent the same procedure but without occlusion of the anterior mesenteric and renal arteries. In the intestinal IPC group (n = 16), the rats were subjected to 3 cycles of 8-min anterior mesenteric artery occlusion followed by 5-min reperfusion without renal IR.

The rats were reanesthetized at the end of the 2- or 24-h reperfusion. The right carotid artery was isolated and cannulated with a polyethylene catheter after a neck incision. Blood samples (6 to 7 ml per rat) were collected for measurement of plasma Cr and BUN. The abdomen was opened, and the kidney was removed and placed in 10% buffered formaldehyde for histopathologic examination or homogenized for measurement of the MDA level and the activities of SOD and CAT. The rats were euthanized by exsanguination immediately after blood and renal samples were obtained.

Determination of plasma concentrations of Cr and BUN. Plasma concentrations of Cr and BUN were measured by using standard diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Determination of the MDA level in renal cortex. The tissue MDA level was determined by the method of Draper and Hadley,[8] which is based on the reaction of MDA with thiobarbituric acid (TBA) at 95 °C. In the TBA test reaction, MDA and TBA react to form a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2 to 3 and 95 °C for 15 min. The sample was mixed with 2.5 volumes of 10% (wt/vol) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation, and 1.0 ml of supernatant was reacted with an equal volume of 0.67% TBA in a boiling waterbath for 15 min. After cooling, the absorbance at 532 nm was determined and expressed in arbitrary units. The values obtained were compared with those from a series of standard solutions (1,1,3,3-tetramethoxypropane). Results were expressed as nanomoles per milligram of protein from tissue.

Table 1. Effect of small intestinal ischemic preconditioning on renal histopathologic scores

	Duration of reperfusion	
	2 h	24 h
Sham	4.6 ± 0.6	5.3 ± 0.8
IPC	5.2 ± 0.7	6.2 ± 0.7
IR	117.7 ± 9.3[a]	186.4 ± 7.2[a,c]
IPC+IR	90.5 ± 5.8[b]	153.6 ± 8.8 [b,c]

IPC, small intestinal ischemic preconditioning; IR, renal ischemia–reperfusion.
Renal histopathology was examined after 2 or 24 h of reperfusion.
All values are expressed as mean ± standard error of the mean (n = 8).
[a]$P < 0.01$ compared with time-matched sham group.
[b]$P < 0.01$ compared with time-matched IR group.
[c]$P < 0.01$ compared with the same group at 2-h reperfusion.

Determination of the activities of SOD in renal cortex. The level of nitroblue tetrazolium (NBT)-reductive substance in the renal cortex, reflecting the activity of SOD, was measured spectrophotometrically as previously described.[32] Activity in the ethanol phase of the lysate was assessed after 1.0 ml ethanol:chloroform mixture (5:3, vol/vol) was added to 1.0 ml of sample and centrifuged, and the absorbance was determined at 560 nm. One unit of SOD was defined as the amount of enzyme that caused 50% inhibition in the NBT reduction rate. SOD activity was expressed as units per milligram of protein from renal tissue.

Determination of the CAT activity in renal cortex. The CAT activity in the renal cortex was measured spectrophotometrically as previously described.[7] The method was based on determination of the rate constant or the H_2O_2 decomposition rate at 240 nm. The extinction was read exactly 1 min after H_2O_2 addition. Results were expressed as k (rate constant) per gram of protein.

Protein assay. The amount of renal cortex homogenate protein was determined using the Bradford method.[2] Coomassie brilliant blue G250 staining reagent (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) was used for protein analysis, with bovine serum albumin prepared as a standard.

Histopathologic analysis. Tissue sections (4 μm) were stained with hematoxylin and eosin and examined under a light microscope (model BX-41, Olympus Optical Co, Ltd, Tokyo, Japan). Lesions were scored according to the following criteria: from each kidney 100 cortical tubules from at least 10 different areas were scored, and care was taken to avoid repeated scoring of different convolutions of the same tubule. Points were given for the presence and extent of tubular epithelial cell flattening (1 point), brush border loss (1 point), cell membrane bleb formation (1 or 2 points), interstitial edema (1 point), cytoplasmic vacuolization (1 point), cell necrosis (1 or 2 points), and tubular lumen obstruction (1 or 2 points).[23] Therefore, scores ranged between the best possible score of 0 (no pathology seen in any of the 100 tubules examined) to the worst possible score of 1000 (maximal pathology seen in all of the 100 tubules examined). An experienced histologist made the assessments in a blinded fashion.

Statistical analysis. All data were expressed as mean ± standard error of the mean. The data were analyzed using one-way analysis of variance and the Newman-Keuls Student *t* test. The threshold for statistical significance was chosen as a *P* value of 0.05.

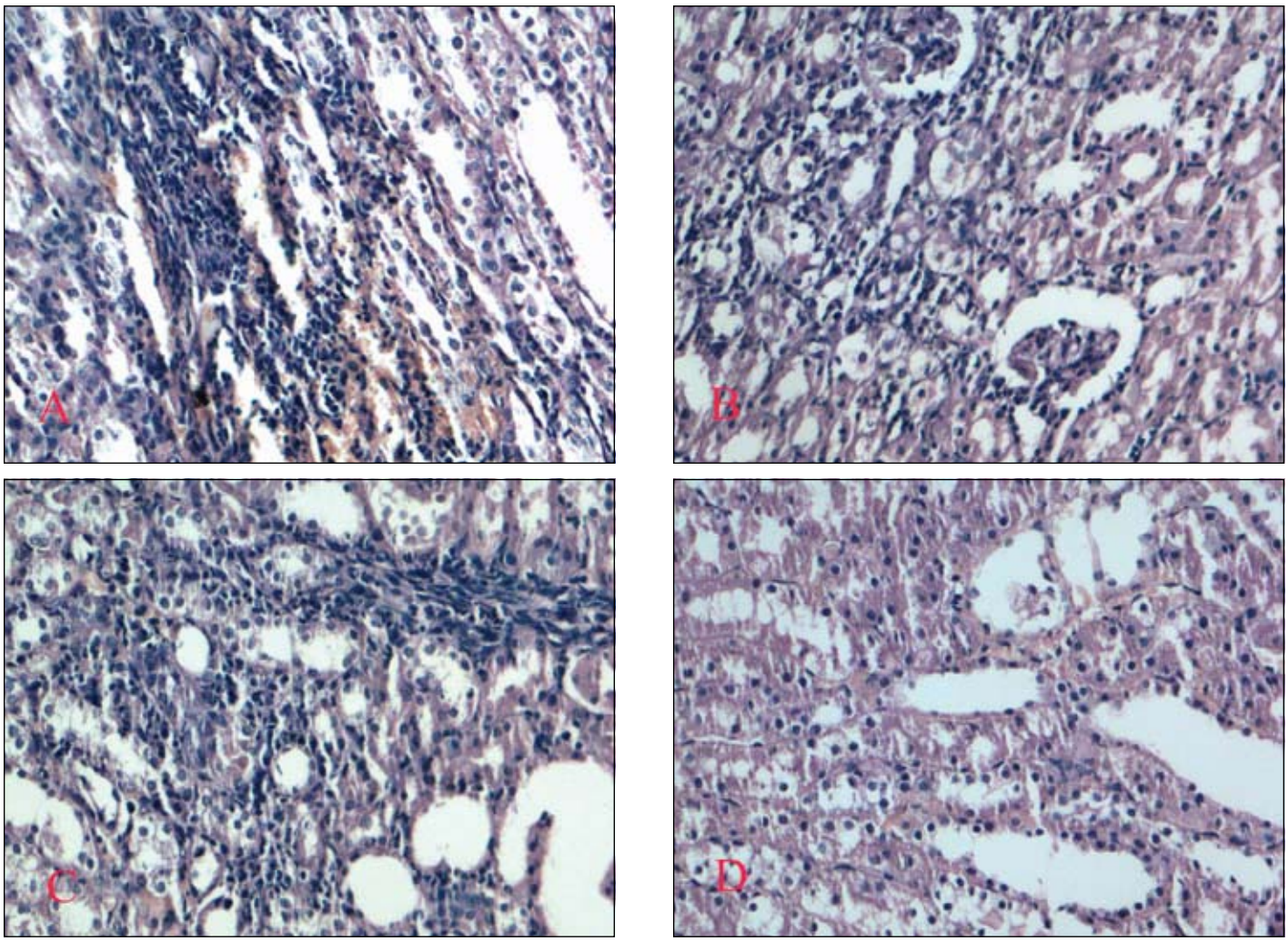

Figure 1. Light microscopy of rat kidney. (A) IR group after 2-h reperfusion, showing severe vascular congestion, hydropic degeneration, and inflammatory cell infiltration. (B) IPC+IR group after 2-h reperfusion, showing mild hydropic degeneration and inflammatory cell infiltration. (C) IR group after 24-h reperfusion, showing marked inflammatory cell infiltration and tubular epithelial cell necrosis. (D) IPC+IR group after 24-h reperfusion, showing tubular dilation and proteinaceous casts. Hematoxylin and eosin stain; magnification, ×200.

Results

Renal histopathologic changes. The histopathologic changes were scored and are summarized in Table 1. Small intestinal IPC itself did not cause any morphologic changes apparent with microscopic observation of rat kidney. Renal IR caused various degrees of vascular congestion, hemorrhage, hydropic degeneration, and inflammatory cell infiltration after 2-h reperfusion, whereas various degrees of tubular degeneration and necrosis, protein cast, and granular degeneration occurred after 24-h reperfusion (Figure 1), scored as 117.7 ± 9.3 and 186.4 ± 7.2, respectively. Pretreatment with small intestinal IPC markedly reduced the lesion scores of kidney after both reperfusion periods compared with that of the IR group.

Plasma concentrations of Cr and BUN. To assess renal function, we measured the concentrations of plasma Cr and BUN. Renal ischemia for 45 min followed by reperfusion resulted in marked increases in Cr and BUN, and renal damage increasingly worsened with prolongation of reperfusion. Pretreatment of small intestinal IPC markedly decreased plasma concentrations of Cr and BUN after both reperfusion periods. Small intestinal IPC itself had no effect on plasma concentrations of Cr and BUN (Figure 2).

MDA levels and activities of SOD and CAT in renal cortex. Renal IR caused a marked increase in the level of MDA and marked decreases in the activities of SOD and CAT in renal cortex. Pretreatment with small intestinal IPC dramatically decreased the level of MDA and increased the activities of SOD and CAT. Small intestinal IPC itself had no effect on the MDA level and the activities of SOD and CAT (Figure 3).

Discussion

rIPC is a new promising field for exploration and may have important clinical implications because it widens the potential practicability of IPC in clinical settings. The present study furnishes, to our knowledge, the first evidence that application of brief small intestinal ischemia (rIPC) attenuates renal IR injury in rat, as shown by decreases in the levels of Cr, BUN, and MDA; attenuation of histopathologic change; and preservation of SOD and CAT activities.

In rats, the severity of IR-induced renal damage depends on the duration of the ischemic insult, in that an ischemic insult of

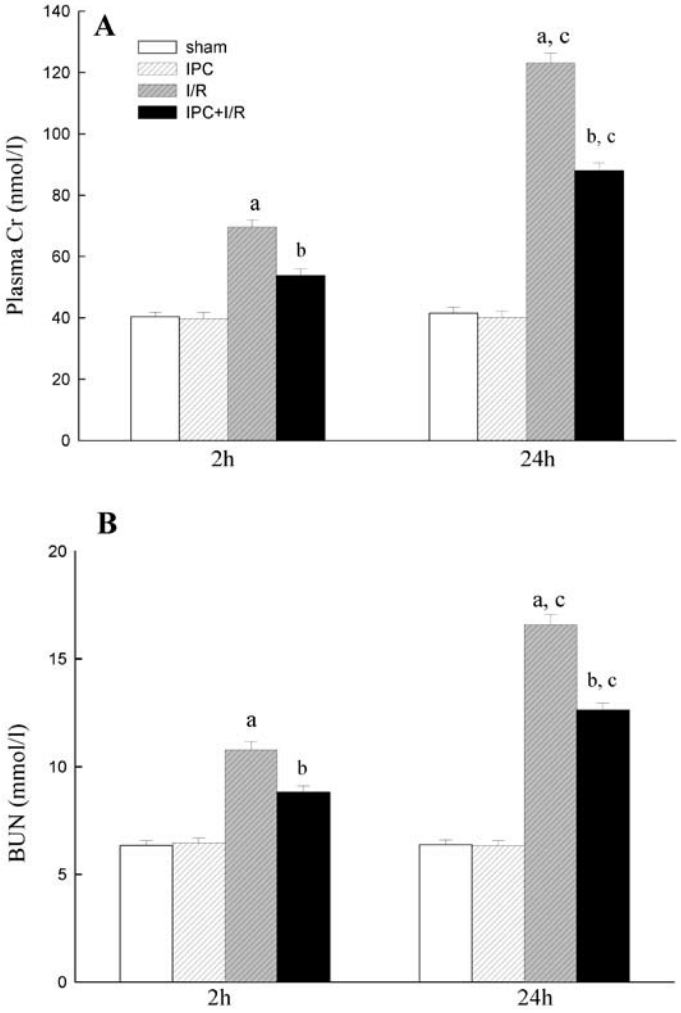

Figure 2. Effects of small intestinal ischemic preconditioning on plasma concentrations of (A) Cr and (B) BUN. IPC, small intestinal ischemic preconditioning; IR, renal ischemia-reperfusion. All values are expressed as mean ± standard error of the mean (n = 8). [a]$P < 0.01$ compared with time-matched sham group; [b]$P < 0.01$ compared with time-matched IR group; [c]$P < 0.01$ compared with the same group at 2-h reperfusion.

less than 30 min causes mild renal injury, 40- to 60-min ischemia causes severe but reversible renal damage,[6] and ischemia for more than 1 h produces irreversible renal injury.[9] Furthermore, it is well documented that kidney impairment, apparent as changes in renal function, after ischemia appears as early as 2 h after reperfusion and reaches its peak 24 h after reperfusion.[38] Therefore, in the present study, we induced renal IR injury by 45-min renal artery occlusion and 2-h or 24-h reperfusion after contralateral nephrectomy. The present results confirmed previous observations[13,16] that IR causes remarkable functional and morphologic renal injury.

Renal ischemia leads to a series of cellular events, which may end with organ failure depending on the duration of blood deprivation. Moreover, reperfusion actually aggravates renal damage.[39] The mechanisms responsible for renal IR injury are not fully understood, but lipid peroxidation—induced by reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-)—has been postulated to play an important role in postischemic acute renal injury.[23] The isch-

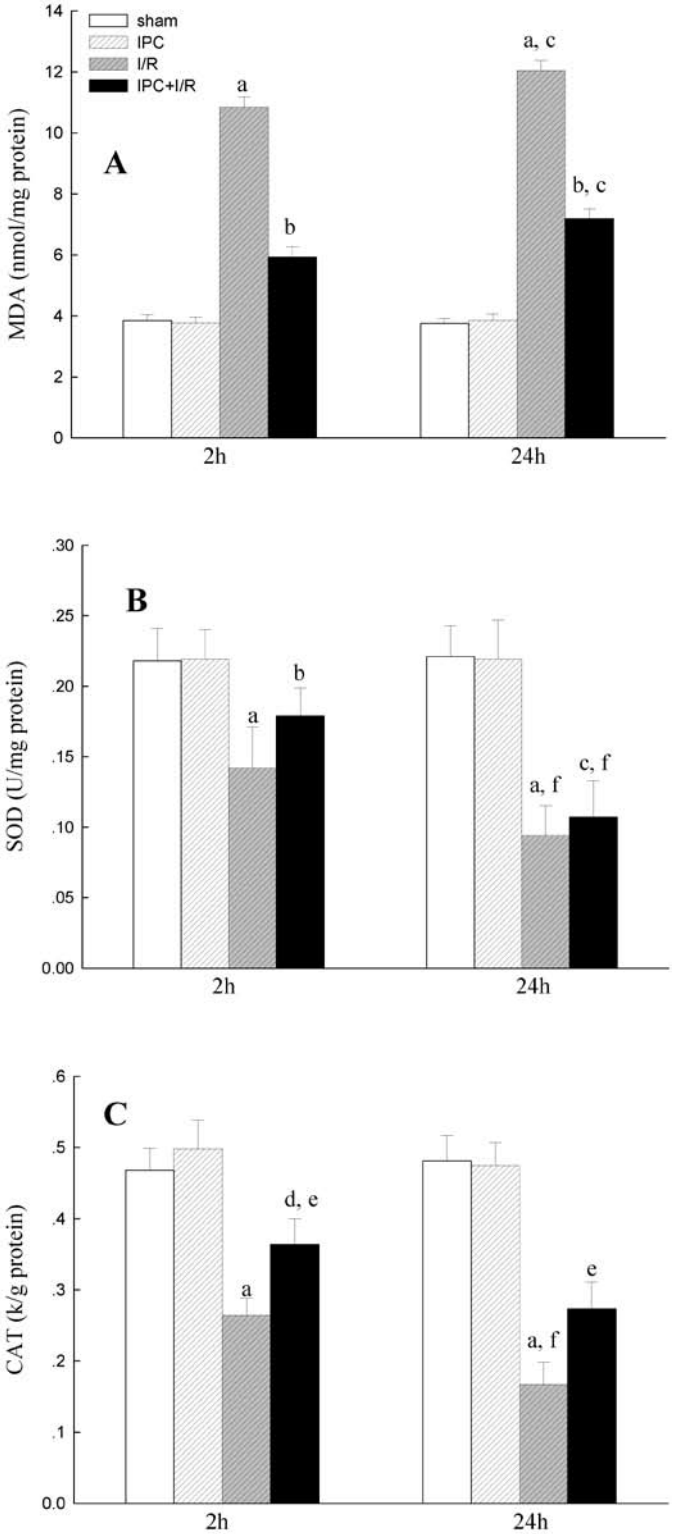

Figure 3. Effects of small intestinal ischemic preconditioning on (A) the level of MDA and the activities of (B) SOD and (C) CAT. IPC, small intestinal ischemic preconditioning; IR, renal ischemia–reperfusion. All values were expressed as mean ± standard error of the mean (n = 8). [a]$P < 0.01$ compared with time-matched sham group; [b]$P < 0.01$ compared with time-matched IR group; [c]$P < 0.01$ compared with the same group after 2-h reperfusion; [d]$P < 0.05$ compared with time-matched sham group; [e]$P < 0.05$ compared with time-matched IR group; [f]$P < 0.05$ compared with the same group after 2-h reperfusion.

emic kidney shows a rapid burst in ROS at the onset of reperfusion, when the oxygen supply is restored.[19] Alternatively, it has been proposed that inflammatory cells, especially neutrophils, that are activated during ischemia, presumably by cytokines, enter the kidney at the onset of reperfusion, causing tissue damage by releasing ROS.[12] The ROS generated during the proposed episodes of IR provoke severe deleterious changes at cellular level leading to cell death. It is known that SOD and catalase CAT are antioxidant enzymes that participate in the detoxification of O_2^- and H_2O_2 in subsequent reactions. The activities of SOD and CAT are reported to gradually decrease during reperfusion.[7]

In the present study, renal IR caused a significant increase in the level of MDA, an end product of lipid peroxidation, and an obvious decrease in the activities of SOD and CAT in renal cortex. Pretreatment with small intestinal IPC significantly decreased the level of MDA and increased the activities of SOD and CAT. These results suggest that rIPC of the small intestine protects against renal IR injury via the inhibition of lipid peroxidation due to preservation of SOD and CAT activities. A reduced level of lipid peroxidation was observed in a study of hepatic preconditioning on renal IR injury,[1] in which brief hepatic ischemia significantly depressed elevation of the MDA level in the ischemic rat kidney after 45-min reperfusion. However, Ogawa and colleagues[22] reported that in the model of 30-min bilateral renal ischemia and 90-min reperfusion, pretreatment with 1 cycle of 5-min bilateral renal ischemia ameliorated renal function and hemodynamics during reperfusion, but this pretreatment had no influence on lipid peroxidation in renal tissue. The possible reason for these differences may be due to the involvement of different mechanisms in the processes of IPC and rIPC of kidney.

The mechanisms underlying IPC have not been well defined, and those of rIPC even less so. It has been speculated that the release of various mediators such as adenosine, bradykinin, and endogenous opioids is triggered by different preconditioning stimuli and that these mediators, which then act on their respective receptors through different signaling pathways, may ultimately lead to activation of phosphokinase C, tyrosine kinase, and p38MAPK kinase and opening of mitochondrial K_{ATP} channels.[24] Further, K_{ATP} channels are assumed to be an effector of preconditioning.[20] However, Lee and colleagues[17] documented that glibenclamide (K_{ATP} channel blocker) fails to abolish and pinacidil (K_{ATP} channel opener) does not mimic IPC protection in rat kidney; these results indicate that IPC protects kidney by a mechanism that is independent of K_{ATP} channel activation. If so, this pathway also is unlikely to account for the mechanism of rIPC in kidney. Beside humoral mediators, neurogenic mechanisms have been implicated as mediators of rIPC in many other investigations.[10,25,33,41] Therefore, whether the renal protective effect of small intestinal IPC is related to humoral or neurogenic mechanisms deserves further investigation.

The small intestine is known as one of the organs most susceptible to ischemia, and IR-induced injury is especially prominent in the intestinal tissue.[4] Moreover, severe intestinal IR injury may result in a systemic response, with damage occurring in remote organs such as the lung,[29] liver,[36] and kidney.[15] It is increasingly recognized that intestinal IR is a primary effector of multiple-system organ failure, and oxidant species and activated neutrophils are presumably the agents responsible for remote organ injury after intestinal IR.[28] However, pretreatment with brief small intestine IR not only elicits a local protective effect[30,42] but also remotely protects heart from later, lethal IR insult.[10,38] Extrapolating from this paradigm, we found that small intestinal IPC similarly protected the kidney in the present study. Although this study does not provide a precise explanation for the observed protection, our findings expand those from observation of hepatic preconditioning on renal IR injury and may have potential clinical application in the protection of kidney against IR injury during surgical procedures in which renal ischemia is unavoidable. Particularly in the case of kidney transplantation, in which the ischemia time of the donor kidney is a leading determinant of graft outcome and patient survival after transplantation, the present results suggest that pretreatment of the living donor with small intestinal IPC prior to procurement would protect the kidney against ischemia and subsequent reperfusion injury.

In conclusion, the results of the current study suggest that small intestinal IPC protects against renal IR injury. These protective effects are related to inhibition of lipid peroxidation and the preservation of SOD and CAT activities.

Acknowledgments

The authors are very grateful to Zhong-Liang Hu (Department of Pathology at Central South University) for his excellent technical assistance.

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