Reactive oxygen species generated by renal ischemia and reperfusion trigger protection against subsequent renal ischemia and reperfusion injury in mice

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Kim J, Jang HS, Park KM. Reactive oxygen species generated by renal ischemia and reperfusion trigger protection against subsequent renal ischemia and reperfusion injury in mice. Am J Physiol Renal Physiol 298: F158–F166, 2010. First published October 28, 2009; doi:10.1152/ajprenal.00474.2009.—Ischemic preconditioning by a single event of ischemia and reperfusion (SIRPC) dramatically protects renal function against ischemia and reperfusion (I/R) induced several weeks later. We recently reported that reactive oxygen species (ROS) and oxidative stress were sustained in a kidney that had functionally recovered from I/R injury, thus suggesting an association between SIRPC and ROS and oxidative stress. However, the role of ROS in SIRPC remains to be clearly elucidated. To assess the involvement of ROS in SIRPC, mice were subjected to SIRPC (30 min of bilateral renal ischemia and 8 days of reperfusion) and then exposed to I/R injury. Thirty minutes of bilateral renal ischemia in the non-SIRPC mice resulted in a marked increase in plasma creatinine levels and removed

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by severe I/R injury, or SIRPC, is associated with redox status. In this study, we determined that SIRPC protected the kidney against I/R and that this protection is triggered by the increased ROS production induced by SIRPC.

MATERIALS AND METHODS

Animal preparation. All experiments were conducted with 8- to 10-wk-old C57BL/6 male mice weighing 20–25 g each. The studies were approved by the Institutional Animal Care and Use Committee of Kyungpook National University. Mice were allowed free access to water and standard mouse chow. Animals were anesthetized with pentobarbital sodium (60 mg/kg body wt ip; Sigma, St. Louis, MO) before surgery. Kidney ischemia was induced as previously described (19, 34). In brief, body temperature was maintained at 36.5–37.5°C. Kidneys were exposed via flank incisions. Mice were subjected to 30 min of either bilateral renal ischemia or reperfusion for 8 days (SIRPC) or sham-operation (non-SIRPC). Ischemia was induced by clamping both renal pedicles with nontraumatic microaneurysm clamps (Roboz Surgical Instruments, Washington, DC). The incisions were temporarily closed during ischemia. After the clamps were removed, reperfusion was confirmed visually. SIRPC or non-SIRPC mice were subjected to 30 min of either bilateral renal ischemia or sham 8 days after reperfusion. Some mice were treated intraperitoneally with either MnTMPyP (5 mg/kg body wt; Calbiochem, San Diego, CA), N-acetylcysteine (Nac; 50 mg/kg body wt, Sigma), or 0.9% saline (vehicle) beginning 48 h after SIRPC (day 2) and continuing 24 h before subsequent I/R or sham treatments. Kidneys were either snap-frozen in liquid nitrogen for enzyme activity measurements and Western blot analysis or perfusion-fixed in PLP (4% paraformaldehyde, 75 mM 1-l-lysine, 10 mM sodium periodate; Sigma) for histological studies 24 h after I/R or sham-operation. To determine histological damages, PLP-fixed kidneys were washed with phosphate-buffered saline (PBS) three times for 5 min each, embedded in paraffin at room temperature, cut into 2-μm sections with a microtome (RM2165; Leica, Bensheim, Germany), and stained with periodic acid-Schiff (PAS).

Histological damage score. Histological damage levels were scored as described previously (15, 21, 22). Briefly, 50 tubules in the outer medullar of kidney were analyzed by using the following score: 0, no damage; 1, mild damage with rounding of epithelial cells and dilated tubular lumen; 2, severe damage with flattened epithelial cells, loss of nuclear staining, dilated lumen, and congestion of lumen; and 3, destroyed tubules with flat epithelial cells lacking nuclear staining and congestion of lumen. Four kidneys from each experimental condition were used (n = 4). Ten fields per slide were used for the counting.

Renal functional parameters. Seventy microliters of blood were obtained from the retrocaval vein plexus at 4, 24, 48, 72, 96, or 192 h after SIRPC or non-SIRPC, and 4 or 24 h after subsequent I/R or sham. Plasma creatinine (PCr) and blood urea nitrogen (BUN) concentrations were measured with a Beckman Analyzer II (Beckman, Fullerton, CA) and BUN kit (Asan Pharm, Seoul, Korea), respectively.

Determination of superoxide production. As described in previous studies (6, 11, 15, 25, 47), in an effort to measure superoxide production, kidneys excised from mice were homogenized immediately in sucrose buffer (0.32 M sucrose, 10 mM Tris–HCl, pH 7.4; Sigma) on ice with a Dounce homogenizer, after which the kidney lysate (100 μl) was moved to a plastic cuvette (Ratiolab, Dreieich, Germany) containing 0.1% nitro blue tetrazolium. The absorbance of the cuvette was immediately measured every 2 min for a total of 30 min at a spectrum wavelength of 560 nm. Nitro blue tetrazolium alone was read to calculate the zero point. Superoxide values were expressed as intensity [optical density (OD)] per gram of protein.

Measurements of lipid peroxidation and hydrogen peroxide. Thiobarbituric acid-reactive substances (Sigma) were determined as a measure of lipid peroxidation. Samples were evaluated for malondialdehyde production via a spectrophotometric assay for thiobarbituric acid-reactive substances (18, 20–22). Hydrogen peroxide (H2O2) levels were measured using the ferric sensitive dye xylonol orange (Sigma). H2O2 oxidizes iron (II) to iron (III) in the presence of sorbitol, which functions as a catalyst. Iron (III) forms a purple complex with xylonol orange, as described previously (18, 20–22).

Western blot analysis. Western blot analyses were conducted as previously described (20, 22, 34). In brief, renal tissue or cell lysates (30–50 μg protein/lane) were separated on 10% or 12% SDS-PAGE gels and then transferred to Immobilon membranes (Millipore, Bedford, MA). The membranes were incubated with anti-MnSOD (1: 2,500 dilution; catalog no. 574596; Calbiochem), -copper-zinc superoxide dismutase (CuZnSOD; 1:2,500 dilution; catalog no. AB1237; Chemicon, Temecula, CA), -catalase (1:20,000 dilution; catalog no. CR2157RP; Fitzgerald, Concord, MA), HSP-27 (HSP-25; 1:2,500 dilution; catalog no. 06-517; Upstate, Lake Placid, NY), iNOS (1: 1,000 dilution; catalog no. 610333; BD Biosciences, San Jose, CA), and β-actin (1:5,000 dilution; catalog no. A5316; Sigma) antibodies overnight at 4°C. After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies against the appropriate primary antibodies (1:5,000 dilution; Vector Laboratories, Burlingame, CA), exposed to Western Lighting Chemiluminescence Reagent (NEL101; PerkinElmer, Boston, MA), and subsequently developed on X-ray film. The area of each band was analyzed using LabWorks 4.5 software (UVP, Upland, CA).

Fig. 1. Plasma creatinine (PCr; A) and blood urea nitrogen (BUN; B) concentrations after ischemia and reperfusion (IR). C57BL/6 male mice were subjected to 30 min of bilateral ischemia and 8 days of reperfusion (SIRPC) or sham surgery (non-SIRPC), then exposed to 30 min of bilateral renal ischemia and reperfusion on day 8. The concentrations of PCr and BUN were measured at 4, 24, 48, 72, 96, or 192 h after SIRPC or non-SIRPC and 4 or 24 h after subsequent I/R or sham. The arrow indicates I/R or sham-operated time. Values are means ± SE; n = 6. *P < 0.05 vs. respective day 0. #P < 0.05 vs. respective sham. SP < 0.05 vs. respective 24 h after SIRPC.
Enzyme activity assay. Tissues in sucrose buffer were homogenized on ice with a Dounce homogenizer. The homogenates were centrifuged for 5 min at 1,000 g, and the supernatants were subsequently centrifuged for 30 min at 15,000 g. The supernatants were used as the cytosolic fraction. The precipitates were washed twice in sucrose buffer to collect mitochondria-containing pellets. The pellets were then resuspended in PBS containing 0.1% Triton X-100, disrupted four times with a sonicator (catalog no. 4710 Series; Cole-Palmer, Chicago, IL) at 40% of the maximum setting for 10 s, and centrifuged for 30 min at 15,000 g (18, 20–22). Catalase activity in the cytosolic fraction was measured by the decomposition of H2O2 (Sigma), determined by a decrease in absorbance at 240 nm (18, 21, 22). One unit of SOD activity was defined as the quantity of enzyme required to reduce the superoxide-dependent color change by 50%.

Statistics. The results are expressed as means ± SE. Statistical differences among groups were calculated using ANOVA followed by least-significant difference post hoc comparisons using SPSS 12.0. Differences between groups were considered statistically significant at a P value of < 0.05. Each group consisted of more than four mice, as indicated in the figure legends.

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Fig. 2. Levels of superoxide (A), hydrogen peroxide (H2O2; B), and lipid peroxidation (C) in the kidneys after ischemia and reperfusion. C57BL/6 male mice were subjected to 30 min of bilateral ischemia and 8 days of reperfusion (SIRPC) or sham surgery (non-SIRPC), then exposed to 30 min of bilateral renal ischemia and reperfusion on day 8. Levels of superoxide, H2O2, and lipid peroxidation were measured 24 h after subsequent I/R or sham, as described in MATERIALS AND METHODS. Values are means ± SE; n = 4–6. “Normal” indicates the kidneys of mice that were not subjected to any operation. MDA, malondialdehyde. *P < 0.05 vs. normal. #P < 0.05 vs. respective sham. $P < 0.05 vs. respective 4 h after I/R. †P < 0.05 vs. respective non-SIRPC.

Fig. 3. Activities of antioxidant enzymes after ischemia and reperfusion. C57BL/6 male mice were subjected to 30 min of bilateral ischemia and 8 days of reperfusion (SIRPC) or sham surgery (non-SIRPC), and then exposed to 30 min of bilateral renal ischemia and reperfusion on day 8. The kidneys were used to measure the activities of manganese superoxide dismutase (MnSOD; A), copper-zinc superoxide dismutase (CuZnSOD; B), and catalase (C) 4 or 24 h after I/R or sham as described in MATERIALS AND METHODS. Values are means ± SE; n = 6. *P < 0.05 vs. normal. #P < 0.05 vs. respective sham. $P < 0.05 vs. respective 4 h after I/R. †P < 0.05 vs. respective non-SIRPC.
RESULTS

SIRPC protects kidneys against subsequent I/R insult. We have reported previously that 30 min of bilateral renal ischemia and reperfusion resulted in functional and morphological kidney injury, and the kidneys recovered from the injury resisted subsequent severe I/R insult (33, 34). Consistent with these findings, 30 min of bilateral renal ischemia and reperfusion in mice markedly increased PCr and BUN concentrations 4 and 24 h after reperfusion, whereas the sham-operation group evidenced no such increase (Fig. 1, A and B). Eight days after ischemia, the increased PCr and BUN levels returned to almost the levels seen in the basal, sham-operated mice (Fig. 1, A and B). When the recovered kidneys, which are referred to as SIRPC kidneys, were subjected to 30 min of bilateral renal ischemia, PCr and BUN levels were not significantly increased at 4 and 24 h after reperfusion, whereas 30 min of bilateral renal ischemia in the non-SIRPC kidneys dramatically increased PCr and BUN concentrations 4 and 24 h after reperfusion (Fig. 1, A and B). This indicates that SIRPC renders the kidneys resistant to I/R insult 8 days later.

I/R induces oxidative stress in non-SIRPC kidneys, but not in SIRPC kidneys. Thirty minutes of bilateral renal ischemia and 4 or 24 h of reperfusion in the non-SIRPC mice significantly increased the levels of superoxide (Fig. 2A), H_{2}O_{2} (Fig. 2B), and lipid peroxidation (Fig. 2C) in the kidneys, but in the SIRPC kidneys no increases in the levels of superoxide (Fig. 2A), H_{2}O_{2} (Fig. 2B), and lipid peroxidation were noted (Fig. 2C).

Thirty minutes of bilateral renal ischemia in the non-SIRPC kidneys resulted in a significant reduction in the activities of CuZnSOD, MnSOD, and catalase, whereas in the SIRPC kidneys the activities of those enzymes were increased (MnSOD significantly; CuZnSOD and catalase not significantly) compared with those in the sham-operated SIRPC group (Fig. 3, A–C). Accompanying the patterns of enzyme activities, the expression of these enzymes was decreased significantly 4 and 24 h after I/R in the non-SIRPC kidneys (Fig. 4, A–D). By way of contrast, the expression of these enzymes in the SIRPC kidneys were not reduced at 4 and 24 h after I/R; rather, the expression of MnSOD increased dramatically (Fig. 4, A–C).

The basal levels of superoxide formation, H_{2}O_{2}, and lipid peroxidation were significantly higher in the kidneys that had recovered from SIRPC than in the non-SIRPC kidneys (Fig. 2, A–C), along with the lowered activities (Fig. 3, A–C) and expressions (Fig. 4, A–C) of MnSOD, CuZnSOD, and catalase. This finding indicates that ROS/oxidative stress persists continuously after SIRPC and may stimulate the development of resistance in SIRPC kidneys.

MnTMPyP or NAc administration inhibits the kidney resistance conferred by SIRPC. To determine whether the protection observed in SIRPC kidneys is developed by ROS generated by SIRPC, we administered antioxidant reagents, either MnTMPyP, a mimetic of SOD, or NAc, a free radical scavenger, to the mice beginning 2 days after SIRPC and continuing to 24 h before subsequent I/R. MnTMPyP or NAc administration significantly prevented the increase in superoxide production after SIRPC compared with normal and vehicle administration (Fig. 5).

Fig. 4. Expressions of MnSOD (A and B), CuZnSOD (A and C), or catalase (A and D) after I/R injury. C57BL/6 male mice were subjected to 30 min of bilateral ischemia and 8 days of reperfusion (SIRPC) or sham surgery (non-SIRPC), and then exposed to 30 min of bilateral renal ischemia and reperfusion on day 8. The expressions of enzymes were measured via Western blot analysis 4 or 24 h after subsequent I/R or sham. A: expressions of MnSOD, CuZnSOD, or catalase were determined via Western blot analysis. Antibody against β-actin was used as an equal protein-loading marker. B–D: densities of blots were quantified using the LabWorks program. Values are means ± SE; n = 4. *P < 0.05 vs. normal. #P < 0.05 vs. respective sham. $P < 0.05 vs. respective 4 h after I/R. †P < 0.05 vs. respective non-SIRPC.
Additionally, these administrations significantly blunted the postischemic increase in MnSOD expression observed in the SIRPC kidneys (Fig. 6).

Thirty minutes of bilateral renal ischemia in vehicle-administered SIRPC mice did not induce an increase in PCr and BUN concentrations at 24 h after reperfusion, but I/R in MnTMPyP- or NAc-treated SIRPC mice increased significantly (Fig. 7, A and B). These increased PCr and BUN levels in MnTMPyP- or NAc-treated SIRPC mice reached ~48–67% of the levels observed in non-SIRPC mice (Fig. 1). Consistent with these functional data, postischemic tubular cell damages in MnTMPyP- or NAc-treated SIRPC mice increased significantly (Fig. 8, A and B). These findings clearly demonstrate that the resistance of SIRPC kidneys is mediated by SIRPC-induced ROS generation.

MnTMPyP or NAc administration inhibits increase in iNOS expression after SIRPC, but does not inhibit increase in HSP-25 expression. As seen in Fig. 7, the administration of MnTMPyP or NAc to SIRPC mice resulted in a significant increase in PCr levels at 24 h after I/R, but the post-I/R increase in PCr level (1.29 ± 0.11 in SIRPC plus MnTMPyP or 1.38 ± 0.14 mg/dl in SIRPC plus NAc mice) did not reach the increased levels of PCr (2.33 ± 0.18 mg/dl) observed in the non-SIRPC mice (Fig. 1A), thereby suggesting that other factors may be associated with SIRPC. In previous studies (33, 34), we determined that increased HSP-25 and iNOS expressions in SIRPC kidneys render the kidneys more protected. Consistent with the findings of our previous studies, HSP-25 and iNOS levels were found to be significantly higher in the SIRPC kidneys than in the non-SIRPC kidneys (Fig. 9, A–C). The treatment of SIRPC mice with MnTMPyP or NAc did not prevent the increase in HSP-25 expression normally observed after SIRPC (Fig. 9, A and B), whereas it significantly inhibited

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**Fig. 5.** Inhibition of the SIRPC-induced increase of superoxide production by the administration of manganese (III) tetrakis(1-methyl-4-pyridyl) porphyrin (MnTMPyP, a cell-permeable SOD mimetic), N-acetylcysteine (NAc; a ROS scavenger), or 0.9% saline (vehicle). C57BL/6 male mice were subjected to 30 min of bilateral ischemia (SIRPC) on day 0 and were then treated daily with intraperitoneal MnTMPyP, NAc, or 0.9% saline (vehicle) beginning 48 h after SIRPC (day 2) for 6 days. The kidneys were harvested for the analysis of superoxide production 8 days after SIRPC (day 8). The production of superoxide anion was measured 8 days after SIRPC, as described in MATERIALS AND METHODS. OD, optical density. Values are means ± SE; n = 4. *P < 0.05 vs. normal. #P < 0.05 vs. vehicle.

**Fig. 6.** Inhibition of SIRPC-induced postischemic increase of MnSOD expression via the administration of MnTMPyP or NAc. C57BL/6 male mice were subjected to 30 min of bilateral ischemia (SIRPC) on day 0 and then treated intraperitoneally with MnTMPyP, NAc, or vehicle beginning 48 h after SIRPC daily and continuing 24 h before 30 min of bilateral renal ischemia. MnSOD expression was measured by Western blot analysis 24 h after subsequent ischemia or sham in SIRPC mice. The antibody against β-actin was utilized as an equal protein-loading marker. The densities of blots were quantified using the LabWorks program. Values are means ± SE; n = 4. *P < 0.05 vs. respective sham. #P < 0.05 vs. respective vehicle.

**Fig. 7.** Inhibition of SIRPC-induced postischemic protective effects on renal function via the administration of MnTMPyP or NAc, as shown in box plots. C57BL/6 male mice were subjected to 30 min of bilateral ischemia (SIRPC) on day 0 and then treated intraperitoneally with MnTMPyP, NAc, or vehicle beginning 48 h after SIRPC daily, and continuing 24 h before 30 min of bilateral renal ischemia. PCr (A) and BUN (B) were measured 24 h after subsequent I/R or sham in SIRPC mice. Box plots indicate range (error bars), 25–75% range (boxes), and median values (horizontal lines) of PCr in either groups; n = 9. *P < 0.05 vs. respective sham.
the increase in iNOS levels (Fig. 9, A and C). These results indicate that increased iNOS expression in the SIRPC kidneys is associated with ROS, but that the increase in HSP-25 expression is not, thereby suggesting that the resistance of kidneys persisting after the administration of MnTMPyP or NAc may be associated with the expression of iNOS.

**DISCUSSION**

In previous studies, we determined that a single event of bilateral renal ischemia and reperfusion (SIRPC), which causes severe renal functional defects and histological damage, protects the kidneys against subsequent I/R insult, and the degree of protection afforded was dependent on the length of the first ischemia and the interval between the first ischemia and subsequent ischemia (33, 34). Consistent with the results of these previous studies, the findings of the present study demonstrate that SIRPC protects kidney functions against subsequent I/R injury.

In the present study, SIRPC was shown to induce functional renal impairment and increased superoxide, H_2O_2, and lipid peroxidation levels. Eight days after SIRPC, the impaired renal function returned to normal; however, the increased superoxide, H_2O_2, and lipid peroxidation levels did not return to basal levels, and the higher levels persisted. This suggests that increased ROS may contribute to the development of resistance in SIRPC kidneys. In fact, the removal of ROS and oxidative stress by the administration of a SOD mimic or a free radical scavenger to the SIRPC mice from 2 days after SIRPC to 24 h before subsequent I/R prevented the increase in superoxide production and the post-I/R increases in MnSOD expression.
observed in SIRPC mice. Furthermore, these treatments resulted in a post-I/R increase in the PCR concentration, which reached levels of 48–67% of those observed in the non-SIRPC group. These findings clearly demonstrate that the protection of SIRPC kidneys is mediated, at least in part, by the ROS generated by SIRPC. In previous studies, we reported that treatment with MnTMPyP, an antioxidant reagent, did not change the antioxidant enzyme activities; however, it increased postischemic antioxidant enzyme activities (18). Therefore, the post-I/R increase in MnSOD expression in MnTMPyP-treated SIRPC (the increased level was higher than in post-sham surgery in vehicle-treated SIRPC mice, but less than in post-I/R in vehicle-treated SIRPC) may be due to initial MnTMPyP treatment. However, the degree of protection afforded by SIRPC was significantly reduced by the treatment with antioxidants along with the reduction of MnSOD expression. It suggests that MnSOD expression increased by SIRPC contributes to the renoprotection afforded by SIRPC.

Although MIRPC differs from SIRPC with regard to the method, degree, and duration of protection, several reports have proposed that ROS triggers the development of MIRPC (17, 36, 45, 46). Hoshida et al. (13) reported that MIRPC confers protection to the heart and that the levels of oxidized glutathione in the preconditioned regions are high, which suggests that the increased antioxidant capacity reduces I/R injury. In the present study, kidneys subjected to SIRPC exhibited higher levels of superoxide, hydrogen peroxide, and lipid peroxidation, along with reduced activity and expression of antioxidant enzymes. However, I/R in the SIRPC group did not induce additional increases in superoxide, hydrogen peroxide, and lipid peroxidation levels and reductions in antioxidant activity and expression. In the SIRPC group, I/R actually reduced oxidative stress, with a concomitant increase in antioxidant enzyme activities and expressions. This indicates that the protection afforded by SIRPC may be mediated by ROS generated as the result of SIRPC. In fact, the protection of the SIRPC kidneys disappeared when antioxidant reagents were administered during SIRPC. These data clearly demonstrate that ROS triggers the development of resistance in the SIRPC kidney and that the resistance is associated with reduced oxidative stress. In MIRPC, Hoshida et al. reported that postischemic MnSOD activity in the MIRPC myocardium was markedly increased compared with that observed in the non-MIRPC myocardium, whereas CuZnSOD activity did not differ between the groups after ischemia (12). Kevin et al. (17) and Chen et al. (5) reported that MIRPC was abrogated by MnTMPyP and NAc (respectively) in the heart. It has also been reported that MnSOD activation induced by oxidative stress during MIRPC contributes to a protective effect in cardiac tissues (7, 14, 50). Recently, we found that kidneys that were exposed previously to irradiation were less susceptible to I/R injury and that this protection was triggered by increased superoxide after irradiation (21).

In the present study, ROS scavengers did not entirely block the protection afforded by SIRPC, thus suggesting the involvement of other factors in the observed protection of SIRPC kidneys. We reported previously that the degree of protection induced by SIRPC is directly proportional to the level of HSP-25 expression (34). Therefore, we attempted to determine whether the induction of HSP-25 after SIRPC was triggered by ROS. The increase in HSP-25 expression in the SIRPC kidneys was not prevented by the administration of MnTMPyP or NAc, which indicates that ROS is not a major contributor to the SIRPC-mediated induction of HSP-25. The unchanged HSP-25 expression after MnTMPyP or NAc treatment may be attributed to the remaining protection, which was not blocked by the administration of MnTMPyP and NAc. HSP-25, which has been shown to function as a molecular chaperone, efficiently trapping unfolding proteins in a folding-competent state (10).
protects against oxidative stress, heat stress, or I/R by stabilizing the actin cytoskeleton and attenuating stress kinase activation, apoptosis, and necrotic cell death (21, 27, 28, 35, 37).

Additionally, we assessed the implications of iNOS in the SIRPC. We have previously described the partial involvement of iNOS in this phenomenon. It was shown, via pharmacological and genetic techniques, that under conditions of increased iNOS expression and iNOS inhibition, the protection normally afforded by SIRPC was abolished (33). In a finding consistent with our previous study, we found in the present study that iNOS expression was increased by SIRPC and that the increase in iNOS was blunted by the administration of antioxidant reagents. This suggests that iNOS or nitric oxide (NO) may be intimately involved in the protection conferred by SIRPC and that an increase in iNOS expression in SIRPC may be triggered by ROS generated by SIRPC. In several reports concerning MIRPC, it has been demonstrated that iNOS and NO are involved in MIRPC-mediated protection in the heart (2, 44) and brain (4, 48). Although many experiments have reported that peroxynitrite, a product of NO and superoxide, is a cytotoxic molecule, recent several studies have demonstrated that peroxynitrite plays a role as a vasodilator and inhibitor of platelet aggregation and inflammatory cell adhesion (26, 30, 31), all of which are beneficial to I/R injury. Thus the reduced renoprotection due to reduced iNOS expression may be associated with the reduced beneficial effect of peroxynitrite. Lefer et al. (24) reported that peroxynitrite at a low concentration exerted a cytoprotective effect in myocardial I/R injury.

In conclusion, our present study demonstrates for the first time that the ROS generated by SIRPC triggers renoprotection. Additionally, in the present study we speculate that kidneys experiencing severe injury are far less susceptible to such injuries in the future, because SIRPC itself induces severe functional and morphological damage in the kidneys. Our findings may provide us with a better understanding of the acquired resistance that organs experienced previously in cases of severe injury and ischemic preconditioning.

GRANTS
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DISCLOSURES
No conflicts of interest are declared by the authors.

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