# Role of nitric oxide in resveratrol-induced renal protective effects of ischemic preconditioning

**Vikas Chander, PhD,** and **Kanwaljit Chopra, PhD,** *Chandigarh, India*

***Background:*** **Resveratrol, a natural antioxidant and polyphenol found in red wine and grapes, has been found to pharmacologically precondition the heart through upregulation of nitric oxide (NO). This study was designed to explore the involvement of NO in the renoprotective effect of resveratrol in renal ischemic preconditioning in rat kidney.**
***Methods:*** **Ischemic preconditioning was induced by three cycles 2-minutes of ischemia followed by 5 minutes of reperfusion before 45 minutes of prolonged ischemia. Resveratrol was given 1 hour before the surgical procedures.**
***Results:*** **Ischemic preconditioning and resveratrol treatment significantly improved the renal dysfunction, decrease in total NO levels, and oxidative stress induced by 45 minutes of ischemia followed by 24 hours of reperfusion. Histopatholgic examination of the kidneys of ischemic/reperfusion rats revealed severe renal damage, which was attenuated in both preconditioned and resveratrol-treated animals. Preconditioning and resveratrol administration led to a marked increase in NO levels in kidney. Renoprotective effects of resveratrol were abolished when animals were pretreated with $N^G$-nitro-L-arginine methyl ester, a nonspecific NO synthase inhibitor.**
***Conclusions:*** **These findngs demonstrate an important contributory role of NO in the protection afforded by resveratrol in renal ischemic preconditioning. (J Vasc Surg 2005;42:1198–1205.)**

***Clinical Relevance:*** **It is now well established that brief periods of ischemia followed by reperfusion render a variety of tissues tolerant to subsequent ischemia/reperfusion-induced injury. This phenomenon, referred to as ischemic preconditioning, was first demonstrated in the dog myocardium. The potential for clinical application of such a powerful protective phenomenon has generated enormous interest in identifying the underlying intracellular signaling pathways, with the ultimate aim of pharmacologically exploiting these mechanisms to develop therapeutic strategies that can enhance tolerance to ischemia/reperfusion injury in patients. This study explored the possible involvement of nitric oxide in renal ischemic preconditioning.**

It is now well established that brief periods of ischemia followed by reperfusion render a variety of tissues tolerant to subsequent ischemia/reperfusion (I/R)-induced injury. This phenomenon, referred to as *ischemic preconditioning* (IP) by Murry et al,[1] was first demonstrated in the dog myocardium. Protective effects of IP on I/R injury have subsequently been revealed in other species, including pigs,[2] rabbits,[3] and rats,[4] as well as in other organs, including the rat small intestine,[5] skeletal muscle,[6] liver,[7] and brain.[8] However, evidence supporting a role for IP in protecting against I/R injury in kidney is scant and controversial.[9,10] I/R injury in the kidney causes severe tissue damage with typical functional and pathophysiological characteristics, including diminished glomerular filtration rate, tubular sodium reabsorption, and renal blood flow. Tissue blood flow is dramatically improved in I/R injury of the myocardium and skeletal muscle after IP.[1-4,6] Although the mechanisms of IP have yet to be fully elucidated, activation of vasodilating factors (including adenosine triphosphate–dependent potassium channels, adenosine $A_1$ receptors, or both[11]; nitric oxide [NO][12]; and protein kinase C[13]) and prevention of cell apoptosis through downregulation of caspases[14] are thought to be involved.

NO plays an important role in regulating renal hemodynamics and functions.[15] A great deal of evidence has suggested that NO is generated not only in the renal vascular endothelium, but also in other renal cells, such as mesangium, macula densa, and tubular cells,[16] thereby suggesting that endogenous NO plays an important role in the regulation of renal blood flow, renal perfusion pressure, renal vascular tone, renal tubular reabsorption, and glomerular filtration rate. The role of NO in I/R-induced acute renal failure is controversial.

Resveratrol (*trans*-3,5,4′-trihydroxy stilbene), a naturally occurring phenolic compound, abundantly available in grape skins and in wines, has been found to protect the heart from I/R injury.[17,18] It possesses diverse biochemical and physiological actions, which includes estrogenic, antiplatelet, and anti-inflammatory properties.[19,20] Recently, resveratrol was found to protect kidney, heart, and brain from I/R injury.[17,18,21,22] In kidney cells, resveratrol was found to exert its protective action through upregulation of NO.[22] The ability of resveratrol to stimulate NO production during I/R is believed to play a crucial role in its ability to protect kidney cells from I/R injury.[19,23]

Ray et al[24] have demonstrated the ability of resveratrol to protect the ischemic reperfused myocardium by improving postischemic ventricular function and by attenuating myocardial infarction due to both necrosis and apoptosis. Although the in vivo antioxidant ability of resveratrol is

From the Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University.
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Reprint requests: Kanwaljit Chopra, Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India (e-mail: chandervikas@yahoo.co.in)
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believed to be at least partially responsible for the cardioprotective properties of resveratrol, the mechanism of action is not completely understood. Recently, a study by Imamura et al[25] demonstrated that resveratrol was unable to precondition inducible NO synthase (iNOS) knockout mouse hearts, whereas it could successfully precondition wild-type mouse hearts, thus indicating an essential role of iNOS in resveratrol preconditioning of the heart.

On the basis of these findings, the purpose of this study was to determine whether I/R-induced renal dysfunction and tissue injury can be overcome by IP and to determine whether endogenous NO contributes to the protective effect of resveratrol in preconditioning of the kidney.

## MATERIALS AND METHODS

### Surgical procedure

All protocols were approved by the Institutional Animal Ethical Committee of the Panjab University. The study was performed on male Wistar rats weighing between 200 and 250 g. Animals were anesthetized with thiopental sodium (40 mg/kg intraperitoneally) and placed in the supine position under a heating lamp to maintain the body temperature at 36°C to 37°C. To induce renal ischemia, a midline laparotomy was performed, and the blood supply to both kidneys was interrupted by occluding the renal pedicles. Reflow was initiated by removing the occlusion.

### Drugs

Resveratrol (Sigma, St Louis, Mo) was suspended in 0.5% sodium carboxy methyl cellulose, and $N^G$-nitro-L-arginine methyl ester (L-NAME; Sigma) was dissolved in distilled water.

### Histologic studies

Immediately after the animal was killed, the right kidney was isolated and washed with ice-cold saline. It was then fixed in a 10% neutral buffered formalin solution, embedded in paraffin, and used for histopathologic examination. Five-micrometer-thick sections were cut, deparaffinized, hydrated, and stained with hematoxylin and eosin. The renal sections were examined in blind fashion for tubular cell necrosis, intertubular cell detachment, interstitial edema, and medullary congestion in all treatments. Abnormalities were graded by using a semiquantitative scale from 0 to 4+, in which 0 denotes no abnormalities; 1+, changes affecting less than 25% of the sample; 2+, changes affecting 25% to 50% of the sample; 3+, changes affecting 50% to 75% of the sample; and 4+, changes affecting >75% of the sample.

### Study design

**Design A.** To study the effect of IP in I/R and to obtain the most suitable preconditioning schedule, an experiment was performed with 42 animals classified into 6 groups:

1. Sham-operated control group (n = 6)
2. The ISC group had no preconditioning and 45 minutes of bilateral ischemia followed by 24 hours of reperfusion (n = 6)
3. The Prec 2/5 group had three cycles of 2 minutes of ischemia and 5 minutes of reperfusion (n = 7) and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion
4. The Prec 3/5 group had three cycles of 3 minutes of ischemia and 5 minutes of reperfusion (n = 7) and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion
5. The Prec 4/5 group had three cycles of 4 minutes of ischemia and 5 minutes of reperfusion (n = 8) and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion
6. The Prec 5/5 group had three cycles of 5 minutes of ischemia and 5 minutes of reperfusion (n = 8) and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion

**Design B.** Using the best schedule of preconditioning from design A, that is, 2 minutes of ischemia followed by 5 minutes of reperfusion (Prec 2/5; hereafter named IP), we determined whether resveratrol-induced NO could mediate IP. For this purpose, another set of experiments was performed that included the following groups:

1. The ISC group had 45 minutes of ischemia followed by 24 hours of reperfusion (n = 6)
2. The IP-ISC group had IP plus 45 minutes of ischemia followed by 24 hours of reperfusion (n = 7)
3. The NO-ISC group was administered resveratrol (5 mg/kg by mouth) 30 minutes before surgery and then had 45 minutes of prolonged ischemia followed by 24 hours of reperfusion (n = 6)
4. The NO-IP group was administered resveratrol (5 mg/kg by mouth) 30 minutes before surgery, then IP, and then had 45 minutes of prolonged ischemia followed by 24 hours of reperfusion (n = 8)
5. The NA-IP group had the nonselective NO synthase (NOS) blocker L-NAME (10 mg/kg intraperitoneally) given 30 minutes before surgery, followed by the IP protocol and, finally, 45 minutes of prolonged ischemia before 24 hours of reperfusion (n = 7)
6. The NO-NA-IP group had resveratrol (5 mg/kg by mouth) and the nonselective blocker L-NAME (10 mg/kg intraperitoneally) administered 30 minutes before surgery, then IP, and then 45 minutes of prolonged ischemia followed by 24 hours of reperfusion (n = 6)
7. The NA-ISC group had the nonselective blocker L-NAME (10 mg/kg intraperitoneally) administered 30 minutes before surgery and then had 45 minutes of prolonged ischemia followed by 24 hours of reperfusion (n = 6)

The animals were placed in individual metabolic cages for 24 hours for urine collection. The animals were anesthetized with high doses of thiopentone sodium (60

mg/kg intraperitoneally), and blood was collected in heparinized centrifuge tubes through the abdominal aorta. The blood samples were centrifuged, and plasma was collected. A midline abdominal incision was performed, and both kidneys were isolated. The left kidney was deep-frozen until further enzymatic analysis, whereas the right kidney was stored in 10% formalin for the histologic studies.

### Assessment of renal function

Serum samples were assayed for blood urea nitrogen (BUN) and serum creatinine by using standard diagnostic kits (Span Diagnostics, Gujarat, India).

### Postmitochondrial supernatant preparation and biochemical estimations

After the animals were killed, their kidneys were quickly removed, perfused immediately with ice-cold normal saline, and homogenized in chilled potassium chloride (1.17%) by using a Potter Elvehjem homogenizer. The homogenate was differentially centrifuged to obtain postmitochondrial supernatant, which was used for further enzymatic analysis.

Malondialdehyde, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid–reacting substances (TBARS).[26] The reduced glutathione (GSH) was measured by the method of Jollow et al,[27] and the yellow color developed by the reduction of Ellman's reagent by –SH groups of GSH was read at 412 nm. Catalase activity was assayed by the method of Claiborne,[28] and the rate of decomposition of $H_2O_2$ was followed at 240 nm. The superoxide dismutase (SOD) activity was assessed by the method of Kono.[29] The nitro blue tetrazolium reduction by superoxide anion to blue formazon was followed at 560 nm.

### Tissue and urine nitrate and nitrite measurements

NO production in renal tissue and urine was determined by the standard Total Nitric Oxide Assay kit (Assay Design, Inc). Nitrate was reduced to nitrite by 3 hours of incubation with nitrate reductase in the presence of nicotinamide adenine dinucleotide 3-phosphate and flavin-adenine-dinucleotide. Nitrite was converted into a deep purple azo compound by the addition of Griess reagent. The total nitrite/nitrate concentration was calculated by using the standard of sodium nitrate. Results were expressed as micromoles per liter.

### Statistical analysis

Values are expressed as means ± SEM. One-way analysis of variance followed by the Dunnett *t* test was applied to calculate the statistical significance between various groups. A value of $P < .05$ was considered to be statistically significant.

## RESULTS

**Protective effect of preconditioning on ischemia to establish the optimal preconditioning protocol.** The serum creatinine, BUN, and creatinine clearance profile is shown in Fig 1. Rats from the ISC group presented severe renal failure as compared with sham-operated animals. The serum creatinine and BUN in the Prec 2/5 group were significantly lower than in the ISC group, whereas the Prec 3/5, 4/5, and 5/5 preconditioning schedules did not protect against ischemia, because the serum creatinine and BUN levels in the Prec 5/5 group were similar to those in ISC rats. The creatinine clearance in ISC rats was significantly lower as compared with sham-operated rats, whereas creatinine clearance in the Prec 2/5 and 3/5 groups was significantly improved. The creatinine clearance in the Prec 4/5 and 5/5 groups was similar to that of the ISC group. Among preconditioning groups, rats with three cycles of 2 minutes of IP (Prec 2/5) had the best renal function.

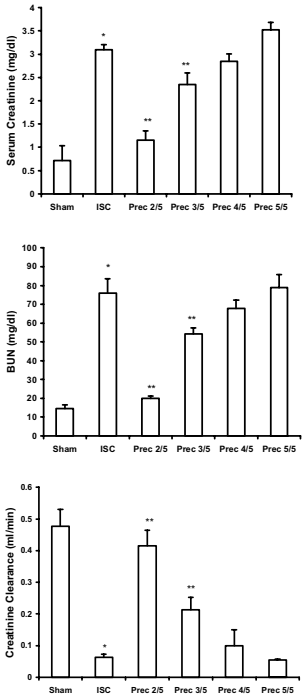


**Fig 1.** Effect of ischemic preconditioning on serum creatinine (A), blood urea nitrogen *(BUN)* (B), and creatinine clearance (C) in ischemic reperfused rats. Values are expressed as mean ± SEM. $*P < .05$ compared with the sham group; $**P < .05$ compared with the ISC group (one-way analysis of variance followed by the Dunnett test). *Prec 2/5*, Three cycles of 2 minutes of ischemia and 5 minutes of reperfusion and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion; *Prec 3/5*, three cycles of 3 minutes of ischemia and 5 minutes of reperfusion and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion; *Prec 4/5*, three cycles of 4 minutes of ischemia and 5 minutes of reperfusion and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion; *Prec 5/5*, three cycles of 5 minutes of ischemia and 5 minutes of reperfusion and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion.

Histologic evaluations revealed severe tubulointerstitial damage in kidneys from the ISC group, whereas only the Prec 2/5 group had a significantly lower degree of histologic damage, as evidenced by lower tubular necrosis, med-

**Table I.** Assay of optimal one-cycle preconditioning schedule based on histologic studies

| *Group* | *Score* |
|---|---|
| Sham | 0.5 ± 0.01 |
| ISC | 3.2 ± 0.5* |
| Prec 2/5 | 0.8 ± 0.02† |
| Prec 3/5 | 2.4 ± 0.2 |
| Prec 4/5 | 3.1 ± 0.5 |
| Prec 5/5 | 3.5 ± 0.4 |

*Prec 2/5*, Three cycles of 2 minutes of ischemia and 5 minutes of reperfusion and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion; *Prec 3/5*, three cycles of 3 minutes of ischemia and 5 minutes of reperfusion and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion; *Prec 4/5*, three cycles of 4 minutes of ischemia and 5 minutes of reperfusion and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion; *Prec 5/5*, three cycles of 5 minutes of ischemia and 5 minutes of reperfusion and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion. Samples were examined for tubular cell necrosis, tubular dilation, intratubular cell detachment, interstitial edema, medullary congestion, and hemorrhage. Abnormalities were graded from 0 to 4+ (0, no abnormalities, 1+, changes affecting <25% of the sample; 2+, changes affecting 25%-50% of the sample; 3+, changes affecting 50%-75% of the sample; 4+, changes affecting >75% of the sample).
*$P < .05$ compared with sham.
†$P < .05$ compared with the ISC group.

ullary congestion and hemorrhage, and development of proteinaceous casts (Table I). The other preconditioning groups showed tubulointerstitial damage similar to that in the ISC group.

**Effect of resveratrol and NOS inhibition on preconditioning.** Treatment with resveratrol in animals subjected to 45 minutes of ischemia followed by 24 hours of reperfusion decreased the serum creatinine and BUN levels and increased the creatinine clearance as compared with the ISC group (Table II). This indicates that resveratrol provides significant protection against dysfunction resulting from ischemia. When NOS was inhibited nonselectively with L-NAME, the effect of preconditioning was abolished, and severe renal failure was observed, with an increase in serum creatinine and BUN and a decrease in creatinine clearance as compared with the ISC group (Table II). Furthermore, three (42.85%) of seven animals from the NA-IP group died because of uremia, thus suggesting that the ischemic damage was also greater. Accordingly, in the group of nonpreconditioned animals receiving L-NAME (the NA-ISC group), comparatively high mortality rates (50%; three of six) and severe renal failure were observed. When resveratrol was added to the group of preconditioned animals receiving L-NAME (the NO-NA-IP group), serum creatinine, BUN, and creatinine clearance levels were similar to those found in preconditioning animals. Histologic evaluation showed marked protection in the NO-ISC, NO-IP, and NO-NA-IP groups as compared with the ISC, NA-IP, and NA-ISC groups (Fig 2; Table III).

**Effect of preconditioning, resveratrol, and L-NAME on biochemical parameters.** Renal I/R (ISC group) produced a significant increase in TBARS levels as compared with sham-operated animals. The TBARS levels were markedly reduced in preconditioning-treated animals (IP-ISC, NO-IP, and NO-IP-ISC groups), and L-NAME–treated animals (NA-ISC and NA-IP groups) showed no decrease in TBARS levels (Table IV).

Renal I/R markedly decreased the enzymatic activity of GSH, catalase, and SOD. This reduction was significantly improved by preconditioning (IP-ISC, NO-IP, and NO-IP-ISC groups), but it was not observed in L-NAME–treated (NA-ISC and NA-IP groups) animals (Table IV).

**Effect of preconditioning, resveratrol, and L-NAME on NO content in urine and preconditioned tissue.** Renal tissue and urine NO, as measured by total NO levels, was significantly lower in the ISC group as compared with sham-operated animals. These levels were significantly improved in the IP group as compared with the ISC group (Fig 3). However, the nonpreconditioned animals that received L-NAME (NA-ISC group) had total NO levels similar to those of the ISC group. These levels were significantly increased in the IP and NO-ISC groups. When resveratrol was added to the group of preconditioned animals receiving L-NAME (NO-NA-IP group), the urine and tissue NO levels were similar to those found in

**Table II.** Effect of resveratrol and nitric oxide synthase inhibition on renal function (plasma creatinine, blood urea nitrogen [BUN], and creatinine clearance) in preconditioned animals

| *Group* | *Plasma creatinine (mg/dL)* | *BUN (mg/dL)* | *Creatinine clearance (mL/min)* |
|---|---|---|---|
| Sham | 0.71 ± 0.32 | 14.38 ± 2.2 | 0.477 ± 0.054 |
| ISC | 3.1 ± 0.103* | 75.87 ± 7.8* | 0.063 ± 0.018* |
| IP-ISC | 1.15 ± 0.21† | 20.01 ± 1.08† | 0.415 ± 0.05† |
| NO-ISC | 1.12 ± 0.25† | 15.59 ± 1.81† | 0.436 ± 0.059† |
| NO-IP | 0.69 ± 0.19† | 12.25 ± 1.09† | 0.485 ± 0.06† |
| NA-IP | 2.65 ± 0.62 | 69.32 ± 5.87 | 0.058 ± 0.012 |
| NO-NA-IP | 1.36 ± 0.26† | 17.62 ± 2.06† | 0.396 ± 0.056† |
| NA-ISC | 3.81 ± 0.1 | 82.25 ± 8.9 | 0.050 ± 0.01 |

Values are expressed as mean ± SEM.
*ISC*, Ischemic group; *IP-ISC*, preconditioned group; *NO-ISC*, resveratrol + ischemic group; *NO-IP*, resveratrol + preconditioned group; *NA-IP*, L-NAME + preconditioned group; *NO-NA-IP*, resveratrol + L-NAME + preconditioned group; *NA-ISC*, L-NAME + ischemic group.
*$P < .05$ compared with sham.
†$P < .05$ compared with the ISC group (one-way analysis of variance followed by the Dunnett test).

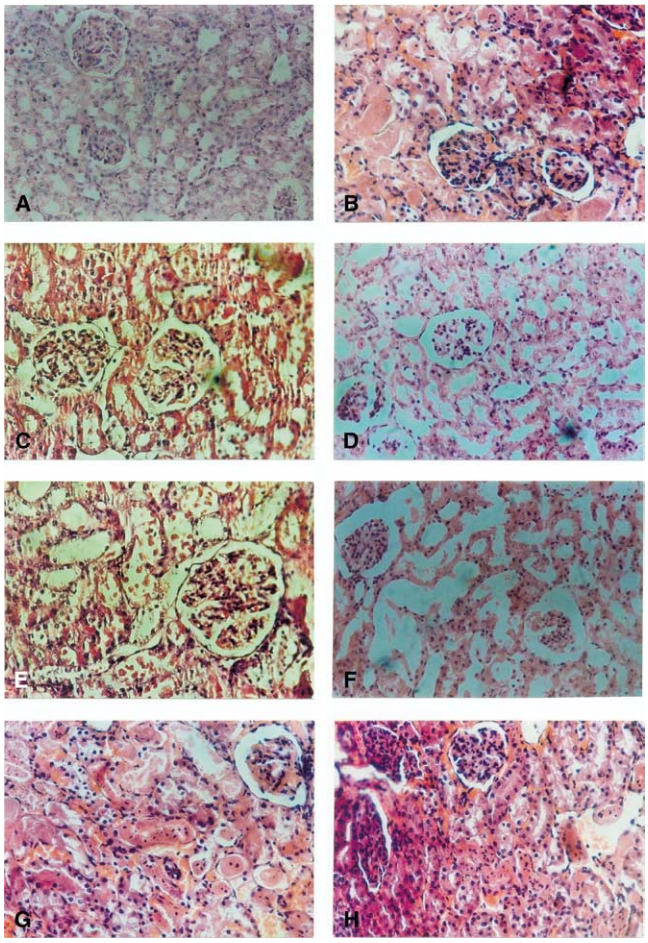


**Fig 2.** Hematoxylin and eosin–stained sections of rat kidneys. A, Renal cortex of rats from the sham group showing normal glomeruli and normal tubules. B, Renal cortex of ISC rats showing severe tubulointerstitial damage, hyaline casts, and medullary congestion. C, Kidney section of a rat treated with the Prec 2/5 protocol (three cycles of 2 minutes of ischemia and 5 minutes of reperfusion and 45 minutes of prolonged ischemia followed by 24 hours of reperfusion) showing only mild medullary congestion. D, Kidney section of a rat treated with resveratrol and ISC showing only mild tubular damage. E, Kidney section of rats treated with resveratrol in the ischemic preconditioning (IP) group showing almost normal morphology. F, Kidney section of rats treated with $N^G$-nitro-L-arginine methyl ester (L-NAME) in the IP group showing severe tubular damage, cast formation, and medullary congestion. G, Kidney section of rats treated with resveratrol and L-NAME in the IP group showing moderate tubular damage, severe medullary congestion, and cast formation. H, Kidney section of rats treated with L-NAME in the ISC group showing severe tubular damage, cast formation, and medullary congestion.

preconditioning animals (Fig 3), thus indicating that resveratrol exerted its effect through the release of NO.

## DISCUSSION

Preconditioning is a simple and harmless method used to render an organ tolerant to ischemia.[30] In this study, we examined whether the three-cycle schedule of preconditioning attenuated the damage induced by sustained ischemia in the kidney and then determined the optimal time window of ischemia and reperfusion. This study demonstrates that three short periods of 2-minute ischemia protect the kidney from subsequent prolonged ischemic insult by a mechanism involving NO. We have also shown that resveratrol protects against ischemic insult through an NO-dependent mechanism.

The destructive effects of I/R inflict direct tissue damage and initiate a cascade of deleterious cellular responses that lead to inflammation, cell death, and organ failure.[31,32] Renal I/R injury secondary to prolonged cessation of blood flow is a significant and common clinical concern.[33,34] Surgical procedures involving the aorta and renal arteries (suprarenal and juxtarenal abdominal aortic aneurysms and renal transplantation, in particular) result in significant postoperative renal complications in the form of acute tubular necrosis and acute renal failure.[33] Therefore, ways to prevent renal dysfunc-

**Table III.** Effect of resveratrol and nitric oxide synthase inhibition on morphologic changes in ischemic preconditioned animals

| *Group* | *Tubular cell swelling* | *Interstitial edema* | *Tubular dilatation* | *Necrosis of epithelium* | *Hyaline casts* |
|---|---|---|---|---|---|
| Sham | — | — | — | — | — |
| ISC | +++ | +++ | +++ | +++ | +++ |
| IP-ISC | — | — | + | — | — |
| NO-ISC | — | — | + | — | — |
| NO-IP | — | — | — | — | — |
| NA-IP | +++ | ++ | + | +++ | + |
| NO-NA-IP | + | + | — | + | — |
| NA-ISC | +++ | +++ | +++ | +++ | +++ |

+, Mild; ++, moderate; +++, severe; *ISC*, ischemic group; *IP-ISC*, preconditioned group; *NO-ISC*, resveratrol + ischemic group; *NO-IP*, resveratrol + preconditioned group; *NA-IP*, L-NAME + preconditioned group; *NO-NA-IP*, resveratrol + L-NAME + preconditioned group; *NA-ISC*, L-NAME + ischemic group.

**Table IV.** Effect of resveratrol and nitric oxide synthase inhibition on thiobarbituric acid–reacting substances (TBARS) and renal oxidative stress in preconditioned animals

| *Group* | *TBARS (nmol/mg protein)* | *GSH (mol × $10^{-4}$)* | *Catalase (k/min)* | *SOD (U/mg protein)* |
|---|---|---|---|---|
| Sham | 36.8 ± 4.12 | 7.89 ± 0.68 | 0.39 ± 0.025 | 8.56 ± 0.43 |
| ISC | 95.2 ± 9.23* | 4.93 ± 0.257* | 0.054 ± 0.008* | 2.59 ± 0.28* |
| IP-ISC | 47.58 ± 8.66† | 7.01 ± 0.52† | 0.32 ± 0.017† | 7.12 ± 0.62† |
| NO-ISC | 38.25 ± 3.38† | 7.65 ± 0.43† | 0.38 ± 0.02 † | 7.85 ± 0.59† |
| NO-IP | 32.69 ± 3.81† | 8.59 ± 0.99† | 0.42 ± 0.021† | 8.37 ± 0.51† |
| NA-IP | 92.83 ± 8.86 | 4.52 ± 0.36 | 0.051 ± 0.007 | 2.12 ± 0.19 |
| NO-NA-IP | 49.12 ± 5.35† | 7.19 ± 0.49† | 0.301 ± 0.011† | 6.9 ± 0.54† |
| NA-ISC | 99.82 ± 10.26 | 4.28 ± 0.29 | 0.058 ± 0.007 | 2.25 ± 0.13 |

Values are expressed as mean ± SEM.
*GSH*, Reduced glutathione; *SOD*, superoxide dismutase; *ISC*, ischemic group; *IP-ISC*, preconditioned group; *NO-ISC*, resveratrol + ischemic group; *NO-IP*, resveratrol + preconditioned group; *NA-IP*, L-NAME + preconditioned group; *NO-NA-IP*, resveratrol + L-NAME + preconditioned group; *NA-ISC*, L-NAME + ischemic group.
*$P < .05$ compared with sham.
†$P < .05$ compared with the ISC group (one-way analysis of variance followed by the Dunnett test).

tion after ischemic manipulation have been the topics of intense research interest.

The importance of reperfusion injury has several implications in clinical practice, and these results indicate that IP may have a role in reducing renal injury during transplantation or renal surgery. Many mechanisms for protection from I/R injury induced by IP have been proposed. Underlying this has been the goal of identifying a final common pathway of protection, with possible pharmacotherapeutic implications.

This study demonstrates that acute preconditioning of the kidney is feasible. On the basis of our preliminary studies, it seems that three repetitive cycles of 2 minutes of ischemia are optimal to precondition the rat kidney in vivo. Our finding is in agreement with the findings of Cochrane et al[35]; however, we did not obtain satisfactory effects with the preconditioning protocols used by Jefayri et al[36] or by Lee and Emala.[37] The beneficial effects of renal preconditioning may differ from those for the heart, where the optimal condition for IP seems to be three cycles of 5 minutes of ischemia,[38] which proved ineffective in the kidneys. According to this work, it seems that cycles involving shorter periods of preconditioning ischemia may be optimal to protect against I/R injury in the kidney.

Signaling mechanisms underlying the NO-mediated IP process have been recently discussed. In isolated rat heart, Lochner et al[39] indicated the importance of the NO/guanylyl cyclase/cyclic guanosine monophosphate pathway in the cardioprotective effects of IP, by using NO donors and inhibitors of NOS and guanylyl cyclase. It has been demonstrated that the activation and translocation of protein kinase C during the cardioprotective effects of IP is NO dependent.[40] The mitochondrial adenosine triphosphate–sensitive $K^+$ channel, which contributes to the IP-mediated myocardial protection,[11] could be activated by NO donors in ventricular myocytes.[41] In addition, it has been reported that hepatic IP is mediated by the inhibitory action of NO on endothelin 1 overproduction induced by I/R.[7]

The study by Yoshida et al[42] clearly demonstrated that resveratrol preconditions rat hearts against lethal I/R injury. The anti-ischemic effect of the resveratrol was blocked by L-NAME, an inhibitor of NO synthesis, thus indicating that NO is the mediator of resveratrol preconditioning of the heart. Recently, iNOS has been demonstrated in resveratrol-induced preconditioning of the heart.[25] Several other recent articles have indicated that preconditioning leads to protection in the kidney against I/R injury, and

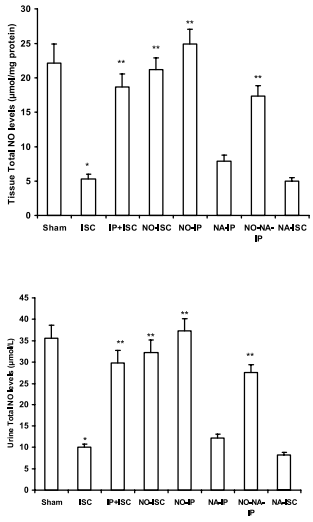


**Fig 3.** Effect of ischemic preconditioning (IP) on tissue (A) and urine (B) total nitric oxide *(NO)* levels. Values are expressed as mean ± SEM. $*P < .05$ compared with the sham group; $**P < .05$ compared with the ISC group (one-way analysis of variance followed by the Dunnett test). *ISC*, Ischemic group; *IP+ISC*, preconditioned group; *NO-ISC*, resveratrol + ischemic group; *NO-IP*, resveratrol + preconditioned group; *NA-IP*, L-NAME + preconditioned group; *NO-NA-IP*, resveratrol + L-NAME + preconditioned group; *NA-ISC*, L-NAME + ischemic group.

this effect was blocked by the nonspecific NO inhibitor L-NAME, thus indicating the involvement of NO in the protective effect of preconditioning in kidney.[43-45] Although resveratrol can scavenge peroxyl radicals in vitro, it is generally known as a poor antioxidant.[18] However, in vivo, resveratrol functions as an antioxidant and decreases the amount of oxidative stress developed in the ischemic reperfused myocardium.[16,25] This seemingly different behavior of resveratrol can be explained if indeed this compound functions through the augmentation of NO. Numerous articles exist in the literature demonstrating massive production of reactive oxygen species in the ischemic reperfused myocardium. NO can readily react with the superoxide anion to form the highly reactive peroxynitrite radical $ONOO^-$, which in turn should preferentially react with the thiols and ascorbates, which are also present in the heart and kidney. In this regard, NO can function as an in vivo antioxidant; hence, resveratrol, through NO, can also function as a potent in vivo antioxidant.

In this study, renal I/R-induced functional and morphologic damage was significantly improved by preconditioning of the kidney and also by prior treatment with resveratrol. It is interesting to note that the renal lipid peroxidation levels were markedly reduced by the IP protocols. Furthermore, the levels of antioxidant enzymes (GSH, catalase, and SOD) were significantly improved by IP and resveratrol. The urinary and serum NO levels, which were markedly decreased by I/R, were found to be significantly improved by IP and prior treatment with resveratrol. Pretreatment with the nonselective NOS inhibitor L-NAME attenuated the protective effect of IP, as well as that of resveratrol. Histopathologic studies have shown a marked protection in the Prec 2/5 group and in the animals pretreated with resveratrol. However, prior treatment with L-NAME abolished the protective effect of resveratrol in ischemic (ISC) animals, and when L-NAME was given along with resveratrol in the IP group, the morphologic protection afforded was similar to that in IP-treated animals. This clearly indicates that resveratrol exerts its protective effect through the release of NO and also through its antioxidant mechanism.

In conclusion, these findings suggest a marked protective effect of IP on I/R-induced acute renal failure and demonstrate the role of NO in resveratrol-induced renal IP.

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