Losartan and Sodium Nitroprusside Effectively Protect against Renal Impairments after Ischemia and Reperfusion in Rats

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Ischemia and subsequent reperfusion are known to impair renal function. We examined several agents that might prevent renal impairment or enhance the recovery of renal function after ischemia/reperfusion injury in rats. Different degrees of preventive effects were observed in rats treated with captopril, BQ-123 (endothelin type A receptor antagonist), sodium nitroprusside (SNP, a nitric oxide donor), and losartan (angiotensin II type 1 receptor antagonist). Only minimal changes in renal morphology were observed after treatment with losartan, SNP, captopril, and BQ-123 compared with control animals. On the other hand, lesions were prominent in the N^3-nitro-l-arginine-methyl ester (l-NAME) and l-arginine-treated rats. The Na^+-K^+ ATPase activity of ischemic kidneys was, however, preserved in all treatment groups, except in those treated with l-arginine and l-NAME, which showed a marked reduction in Na^+-K^+ ATPase activity. Our post-treatment data suggest that losartan and SNP have the greatest potential for therapeutic use to mitigate post-ischemic renal damage and functional impairment.

Key words acute renal failure; angiotensin II; endothelin; nitric oxide; losartan

Ischemic acute kidney injury (AKI) can occur in several common clinical situations, such as severe cardiac failure, generalized sepsis or excessive loss of blood during surgery or due to trauma. This incidence of AKI is much more common than the end-stage kidney disease and is increasing globally. The recovery of renal function after an ischemic acute renal injury is an important clinical determinant of patient morbidity and mortality. It was found that the delayed recovery after AKI was associated with the progression to chronic kidney disease (CKD) and end-stage renal disease.

The decrease in renal blood flow as a consequence of an increase in the renal vascular resistance after ischemia/reperfusion (I/R) exerts a major impact on the degree and rate of recovery of the kidney functions. Therefore, the prevention of an increase in the renal vascular resistance would enhance the recovery of the kidney functions and reduce the negative outcomes of AKI.

There are several substances that have been shown to affect renal vascular resistance in ischemic AKI including nitric oxide (NO), angiotensin II (Ang II) and endothelin. They have been reported to modulate renal vascular resistance and to play important role in an ischemic acute renal injury. In a normal kidney, NO affects both of the renal hemodynamics and renal tubular functions. It decreases both afferent and efferent arteriolar resistances and decreases sodium reabsorption in the renal proximal tubules by reduction of proximal tubule Na^+-K^+ ATPase activity. However, the role of nitric oxide in an acute ischemic renal injury is still controversial. In ischemic AKI, Ang II and renin–angiotensin–aldosterone system (RAAS) can deteriorate the kidney functions by increasing systemic vascular resistance and inflammatory processes. Several studies have shown that inhibition of either Ang II production or action of angiotensin could prevent renal injury in ischemic AKI. The endothelin system is thought to act in an autocrine manner to affect renal hemodynamics and functions. Endothelin receptors A and B are found throughout the kidney. In normal kidney, endothelin-1 (ET-1) plays an important role in modulating vascular smooth muscle resistance and its production was increased in ischemic kidney. The deletion of ET-1 gene or the blockade of endothelin A receptor showed a dramatic protection of renal functions in ischemic AKI.

Despite an enormous effort has been made to determine the protective roles of Ang II inhibitor, endothelin inhibitor, NO donor and NO inhibitor, their effectiveness are still not conclusive. This is, in part, due to different protocols and experimental designs were employed in these studies. Therefore, in the present study using the same protocol and experimental designed, we compared the effectiveness of Ang II inhibitor, endothelin inhibitor, NO donor, and NO inhibitor in protecting against ischemia-reperfused kidneys before and after ischemic insults (pre- and post-ischemic treatments) so as to clarify the mechanisms of the protection. The results obtained may provide important information for developing effective therapeutic strategies for the treatment of ischemic AKI.

MATERIALS AND METHODS

Materials All chemicals used, namely inulin, anthrone, para-amino hippurate (PAH), l-arginine, NO^3- -nitro-l-arginine-methyl ester (l-NAME), sodium nitroprusside (SNP), losartan (COZAAR®), BQ-123, captopril, sodium and potassium standard solutions for flame photometer, were of the analytical grade and were purchased from Sigma Chemical Co., U.S.A., Merck Sharp & Dohme Ltd., Tocris Cookson Inc., U.S.A., Fluka Laborchemikalien GmbH & Co., KG., and Ciba Corning Diagnostics GmbH, Germany. Nitric oxide (NO_2/NO_3) assay kit (Catalog Number DE1500) was purchased from R&D Systems, Inc., U.S.A. Other substances not mentioned were of the highest grade available from many sources.

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Animal Preparation  Male Wistar rats (250–300 g, National Laboratory Animal Center (NLAC), Thailand) were used as an experimental model. All procedures used in the study were approved by the Animal Care and Use Committee of the Faculty of Science, Mahidol University, and performed in accordance with the ethical guidelines of The National Research Council of Thailand. Rats were anaesthetized by an intraperitoneal (i.p.) injection of pentobarbital sodium at a dose of 50 mg/kg body weight (BW). During surgical preparation, the rat’s body temperature was maintained at 37°C. A tracheostomy was performed to facilitate breathing. The right femoral artery was cannulated for blood sample collections and mean arterial blood pressure (MAP) was measured using Statham strain gauge transducer and the results were recorded in Power Macintosh computer (6100/60). The right femoral vein was also cannulated for infusion of 0.9% sodium chloride solution. Both ureters were catheterized for urine collections.

Experimental Procedures Following animal preparation, a solution of 0.9% normal saline (NSS) containing inulin mixed with PAH was given to the rats at an infusion rate of 97 µL/min. After the animals were allowed to equilibrate for about 50 min, two urine samples (20-min interval) were collected from both ureters into small pre-weighed tubes covered with saturated mineral oil. Two blood samples (0.40 mL) were also collected at 20-min interval. The state “T0” represents the mean value of two control periods of both urine and blood samples before ischemic induction. Then, unilateral renal ischemia was induced by left renal artery ligation for 20 min. The right kidney of the same animal was assigned as non-ischemic kidney). After 20 min of ischemic induction, reperfusion was allowed by releasing the ligation. Then, the urine and blood samples were collected during reperfusion period at 20-min intervals for 100 min (T1–T5; collected at 20 min, 40 min, 60 min, 80 min and 100 min after reperfusion, respectively). At the end of the experiment, rats were sacrificed and both kidneys were removed, decapsulated, blotted dry and weighed. The kidneys were processed for a histopathological study. For Na⁺–K⁺ ATPase activity measurement, cortical membranes were prepared from a separate group of animals at the end of the experiment.

Experimental Groups The rats were divided into 9 groups including: 1) unilateral I/R injury without treatment, 2) unilateral I/R with L-arginine pre-treatment (50 mg/kg, intravenous (i.v.) bolus injection, 40 min before I/R induction), 3) unilateral I/R with L-arginine and L-NAME pre-treatment (0.50 mg/kg, i.v. bolus injection, 40 min before I/R induction), 4) unilateral I/R with SNP pre-treatment (5 mg/kg, i.v. bolus injection, 40 min before I/R induction), 5) unilateral I/R with SNP post-treatment (10 µg/kg/min, i.v. continuously infused throughout the reperfusion period), 6) unilateral I/R with losartan treatment (25 mg/kg, subcutaneous (s.c.) injection at the time when ischemic induction was started), 7) unilateral I/R with captopril pre-treatment (1 mg/kg BW, i.v. bolus injection, 10 min before I/R induction), 8) unilateral I/R with captopril post-treatment (1 mg/kg BW, i.v. bolus injection when reperfusion was started), and 9) unilateral I/R with BQ-123 post-treatment (0.1 mg/kg/h, i.v. continuously infused throughout the reperfusion period). The doses of the drugs and treatment times in this study were previously used for the assessment of renal I/R injury.⁰¹⁻⁰⁶,¹⁷⁻²⁵

Renal Function Studies  Urine samples were collected as previously mentioned and urine volume was determined by the difference between pre-weighed and post-weighed tubes. The values obtained from two control samples were combined to obtain an average value (T0). The inulin clearance and PAH clearance were used to estimate the glomerular filtration rate (GFR) and the renal plasma flow (RPF), respectively.²⁶⁻²⁷ Urine flow rate and fractional excretion of Na⁺ (FeNa), which was calculated from urine sodium divided by plasma sodium, were used as indicators of tubular function. Sodium concentrations in both urine and plasma samples were determined by atomic absorption spectrophotometry.

Measurement of Nitrate (NO₃⁻) and Nitrite (NO₂⁻)  Urine NO₃⁻ (NO₃⁻+NO₂⁻) concentration was measured by the reduction of urine NO₃⁻ to NO₂⁻ with nitrate reductase enzyme. The NO₃⁻ was generated directly by measuring the magenta-colored azo dye that is formed from NO₃⁻ and the Griess reagent.²⁸⁻²⁹

Determination of Na⁺–K⁺ ATPase Activity of Rat Renal Cortical Suspension Following I/R Injury  At the end of the experiment, thin sheets of renal cortex were cut off and then were homogenized in an ice-cold buffer solution I containing 0.25 M sucrose, 10 mM Tris-base, and 10 mM N-(2-hydroxyethyl) piperazine-N’-2-ethanesulfonic acid (HEPES) (pH 7.4). The homogenate was centrifuged at 10000×g for 1 h at 4°C. The pellet was resuspended in a bicarbonate-buffer solution II containing 0.25 M sucrose, 20 mM Tris-base, 20 mM HEPES and 1 mM MgCl₂ (pH 7.4) and then used to determine Na⁺–K⁺ ATPase activity from the difference in the amount of inorganic phosphate (Pi) liberated by the hydrolysis of ATP in the presence and absence of 2.5 mM ouabain. Pi was measured by a modification of the method of Fiske and Subbarow.³⁰ Enzyme activity was expressed as µmol Pi/min per mg protein. Protein was measured by a modified Lowry method using Folin Phenol reagent.³¹

Histological Examination  At the end of the experiment, the kidneys were taken out, fixed in formalin, and embedded in paraffin. Serial sections were cut at 5 µm intervals and stained with routine hematoxylin and eosin (H&E) stain. Renal pathology associated with acute tubular necrosis was evaluated at a microscopic level. The slides were observed in at least 10 high-power fields (×400). The number of damaged tubules was calculated as percent of total tubule number.

Statistical Analysis  A one-way ANOVA was performed for multiple comparisons. The individual means were subjected to a Fisher’s least significant differences post hoc tests for statistical difference analysis. The differences between the value of left and right kidneys were evaluated by Student’s paired t-test to compare two dependent variables. Statistical significance was defined as the p value was less than 0.05. Results are expressed as means±standard error of the mean (S.E.M.)

RESULTS

Effect of I/R Injury on Renal Function  In an ischemic kidney (left kidney), GFR and RBF were significantly decreased from their control values after induction of ischemia (from 0.99±0.05 to 0.37±0.04 mL/g kidney weight/min and 14.03±2.55 to 3.09±0.57 mL/g kidney weight/min, respectively). After reperfusion, RBF and GFR were slightly improved towards control values (Figs. 1A, 2A). Of note, I/R

also slightly reduced RBF in the non-ischemic (right) kidney by 40% without causing any significant change in GFR (Figs. 1B, 2B). The urine flow rate as normalized by GFR (V/GFR ratio) and FeNa were increased following ischemic induction from their own control prior to ischemic induction by about 5 fold (V/GFR ratio from 0.023±0.004 to 0.098±0.021) and 3.5 fold (FeNa from 2.96±0.56 to 7.95±1.11%), respectively (Figs. 3A, 4A). Of note, I/R also slightly decreased both GFR and RBF in the non-ischemic kidney from their own control values (prior to ischemic induction) (Figs. 1B, 2B), but they did not reach statistical significance.

**Effects of Pretreatments with L-Arginine, L-NAME, and SNP on Renal Function Following I/R Injury** L-Arginine (precursor of NO synthesis), L-NAME (the nonselective nitric oxide synthase (NOS) inhibitor), or SNP (NO donor) were given by a bolus injection (i.v.) 40 min before induction of ischemia from their own control prior to ischemic induction by about 5 fold (V/GFR ratio from 0.023±0.004 to 0.098±0.021) and 3.5 fold (FeNa from 2.96±0.56 to 7.95±1.11%), respectively (Figs. 3A, 4A). Of note, I/R also slightly decreased both GFR and RBF in the non-ischemic kidney from their own control values (prior to ischemic induction) (Figs. 1B, 2B), but they did not reach statistical significance.

The period “T1” and “T5” represents the values at first 20 min and 100 min after reperfusion, respectively. Ischemia was induced by ligation of the left renal artery (20 min) following the control period (T0). After 20 min of ischemia, reperfusion was allowed by releasing the obstruction. The dose of SNP pre-treatment was 5 mg/kg (bolus i.v. injection). Those of SNP and BQ-123 post-treatment were 10 µg/kg/min and 0.1 mg/kg/h, respectively (continuous i.v. infusion). The doses of losartan and captopril were 25 mg/kg s.c. and 1 mg/kg (bolus i.v. injection in both pre- and post-treatment), respectively. Data are expressed as means±S.E.M. (n=7). Statistically significant difference from the corresponding untreated (right kidney, w/o) value of each period is expressed by symbol (*) at p<0.05. The asterisks (”) indicate significant differences from their own control (T0) values (100%) at p<0.05.
reperfusion, and then it increased towards control values. The RBF (Fig. 2A) of the ischemic kidney was also gradually increased during 20–100 min (T1–T5) periods. It returned back to normal 2 h after reperfusion (data not shown). The V/GFR and FeNa (Figs. 3A, 4A) of the ischemic kidney were significantly increased but to a lesser degree compared with other groups and it had a tendency to decrease back to normal. The GFR and RBF of the non-ischemic kidney (Figs. 1B, 2B) were not changed by losartan administration whereas the urine flow rate and FeNa were significantly increased only at the 20 min period.

Captopril, an ACE inhibitor, was given to the rats by a bolus injection (i.v.) 10 min before ischemia (captopril-pre). It did not significantly change the GFR of the ischemic kidney, but significantly increased GFR in the non-ischemic kidney (Fig. 1). The RBF of the ischemic kidney was markedly decreased only in the 20 min period but did not significantly change in non-ischemic kidney (Fig. 2). On the other hand, V/GFR and FeNa of both ischemic and non-ischemic kidneys were increased throughout the reperfusion period (Figs. 3A, B, 4A, B).

When captopril was given by an i.v. infusion at the start of reperfusion (captopril-post), GFR of the ischemic kidney was significantly decreased from the control value, whereas it was significantly increased in the non-ischemic kidney (Fig. 1). The RBF of the ischemic kidney was slightly, but significantly, lower than its control value in the 20 min period (T1), and it returned towards the control value in the 100 min period (T5) (Fig. 2A). The V/GFR rate of both kidneys was increased at T1 period, whereas FeNa of both kidneys was increased from their control values throughout the reperfusion period (Figs. 3, 4).

BQ-123, an endothelin type A receptor antagonist, was given by continuous intravenous infusion during the reperfusion period. The GFR of both ischemic and non-ischemic kidneys did not significantly change from its control value (Fig. 1). The RBF of the ischemic kidney was improved after BQ-123 administration (Fig. 2A). Significant increases of V/GFR and FeNa were observed in both ischemic and non-ischemic kidneys (Figs. 3, 4).

**Plasma NO\textsubscript{x} (NO\textsubscript{2}\textsuperscript{−}+NO\textsubscript{3}\textsuperscript{−}) and Urinary NO\textsubscript{x}/GFR before and after I/R Injury** To gain insight into the role of NO in I/R-induced damages and the possible mechanisms of substances that interfere with NO metabolism, plasma and urinary NO\textsubscript{x} metabolites were measured. Figure 5 shows plasma nitric oxide metabolites (NO\textsubscript{x} = NO\textsubscript{2}\textsuperscript{−}+NO\textsubscript{3}\textsuperscript{−}) at various periods (T0, T1 and T5). After an induction of ischemia and treatment with drugs involving NO production, plasma NO\textsubscript{x} in the 20 min (T1) and 100 min (T5) periods had a tendency to decrease from its own control (T0) value. However, plasma

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**Fig. 2. The Effects of L-Arginine, L-NAME, SNP, Losartan, Captopril, and BQ-123 on RBF Following I/R of Ischemic/Left (A) and Non-ischemic/Right (B) Kidneys**

Conditions are the same as in Fig. 1. Data are expressed as means±S.E.M. (n=7). Statistically significant difference from the corresponding untreated (w/o) value of each period is expressed by symbol (*) at p<0.05. The asterisks (*) indicate significant differences from their own control (T0) values at p<0.05.
NOx of the untreated, L-arginine and L-NAME pre-treated and the SNP post-treated groups were not significantly different from their own control values after ischemic induction. On the other hand, in the SNP pre-treated group, plasma NOx was significantly increased at 20 and 100 min after I/R injury.

Figure 6 shows the urinary excretion of nitric oxide metabolites expressed as UNOxV/GFR after I/R induction in the absence or the presence of various drugs involving NO production of the ischemic (Fig. 6A) and non-ischemic (Fig. 6B) kidneys. As shown, UNOxV/GFR of the untreated group was markedly increased by 20 min and then returned to control value by 100 min after I/R. Such an increase was also seen in the groups treated with drugs that interfere with NO production, except only in the groups pre-treated with L-arginine and L-NAME. Both L-arginine and L-NAME significantly decreased Na+/K+ ATPase activity from its own non-ischemic (right) kidney and from the ischemic kidney of the untreated group.

Effects of Drugs on Na+/K+ ATPase Activity of Rat Renal Cortical Suspension Following I/R Injury

Table 1 illustrates the Na+/K+ ATPase activity in the renal cortical suspension after I/R injury of both untreated and drug treated groups. In the absence of drug treatment, I/R injury had virtually no effect on cortical Na+/K+ ATPase activity of the ischemic (left) kidney, i.e., 65.68±5.05 μmol Pi/mg protein/min compared to 64.92±7.81 μmol Pi/mg protein/min in the non-ischemic (right) kidney. Treatments with the drugs affecting NO production did not alter the activity of Na+/K+ ATPase except only in the groups pre-treated with L-arginine and L-NAME. Both L-arginine and L-NAME significantly decreased Na+/K+ ATPase activity from its own non-ischemic (right) kidney and from the ischemic kidney of the untreated group.

Renal Histopathological Changes Following I/R Injury

The histological appearance of the kidney was observed by light microscopy. Non-ischemic (right) kidney in the untreated group exhibited normal histology (Fig. 7A) whereas pathological changes were observed in the ischemic (left) kidney in which acute tubular necrosis of the proximal tubular cells characterized by the disruption and detachment of apical cells and brush border were evident (Fig. 7B). The accumulation of proteinaceous materials and cell debris was found in the tubular lumen. The degenerated proximal tubular cells were markedly hydropic (vacuolar) and their nuclei were disappeared, while the distal convoluted tubule (DCT) cells appeared to be normal. Both proximal convoluted tubule (PCT) and DCT showed slight dilatation. Approximately, a half portion of
Fig. 4. The Effects of l-Arginine, L-NAME, SNP, Losartan, Captopril, and BQ-123 on FeNa Following I/R of Ischemic/Left (A) and Non-ischemic/Right (B) Kidneys

Conditions are the same as in Fig. 1. Data are expressed as means ± S.E.M. (n=7). Statistically significant difference from the corresponding untreated (w/o) value of each period is expressed by symbol (#) at $p<0.05$. The asterisks (*) indicate significant differences from their own control (T0) values at $p<0.05$.

Fig. 5. The Effects of l-Arginine, L-NAME, and SNP on Plasma Nitric Oxide Metabolites ($\text{NO}_x = \text{NO}_2^- + \text{NO}_3^-$) after I/R

The period “T0” represents the mean value of two control periods. The periods “T1” and “T5” represent the values at first 20 min and 100 min after reperfusion, respectively. Ischemia was induced by ligation of the left renal artery (20 min) following the control (T0) period. After 20 min of ischemia, reperfusion was allowed by releasing the obstruction. Conditions are referred to in Methods. Data are expressed as means ± S.E.M. (n=6). Statistically significant differences from their own control (T0) values at $p<0.05$ are expressed by asterisks (*).
renal tissues in the I/R group without any treatment showed an acute tubular necrosis.

Representative ischemic kidney sections from rats with L-arginine pre-treatment (Fig. 7C) and L-NAME pre-treatment (Fig. 7D) showed prominent histopathological changes. The cellularity of glomerulus was markedly decreased. The PCT and DCT were dilated and contained cell debris and proteinaceous materials in the lumen. The microvilli brush borders of PCT were disappeared. The nuclei of PCT were evacuated and absent as well. The changes in these treatment groups seemed worse than those in the untreated group. The histopathological changes of renal cortex of ischemic kidney from SNP pre-treated rats (Fig. 7E) were similar to those of L-arginine pre-treated rats, with approximately 90% of tubular structure being damaged. In contrast, the ischemic kidney of SNP post-treated (Fig. 7F) rats were significantly improved and appeared to be normal compared with other groups, i.e., only slightly tubular dilation of PCT and DCT were observed. Furthermore, the lesions were minimal in the rats treated with losartan, captopril and BQ-123 (Figs. 7G–J). These histopathological data correlated well with Na\textsuperscript{+}–K\textsuperscript{+} ATPase activity in Table 1.

**DISCUSSION**

The aim of this study was to determine the best agent that may help enhancing renal function recovery after unilateral I/R. In addition, the effects of the agents administered before and after the initiation of ischemia on the kidneys were also performed. It was found that recovery of the kidney function following I/R injury was enhanced by treatments with SNP and drugs that reduce Ang II or endothelin activities. Among
drugs used in this study, losartan and SNP post-treatment were shown to have best results in improving renal function after I/R injury.

The present study found that renal I/R injury induced glomerular and renovascular impairments with marked decreases in GFR and RBF accompanied by a rise in FeNa without changes in cortical Na\(^+\)–K\(^+\) ATPase activity. These results might be due to the change in levels of the intrarenal NO and vasoconstrictors (especially Ang II and endothelin) leading to hemodynamic changes. Urinary NO\(_x\), an indirect indicator of intra-renal NO production, was markedly increased in the ischemic kidney after I/R injury. The increment in NO production found in this study is consistent with the study by Saito and Miyagawa.\(^{32}\) Using a NO-selective electrode, they reported an increase in NO release in the rat kidney during I/R. One of the possible sites of NO production might be the renal proximal tubule.\(^{33}\) In our study, it is not known whether an increased NO production occurred at the sites other than proximal tubules or not. Not only does the level of NO change in I/R injury, but also Ang II and endothelin have been reported to be increased. Ang II levels in the ischemic kidneys were significantly elevated following renal ischemia.\(^{23,34}\) In addition, Brooks\(^{35}\) reported that plasma ET-1 level was increased in the patients with acute renal failure as well as in rats after renal ischemia.

The decreases in both GFR and RBF after I/R injury might result from the relatively low NO (vasodilator) activity compared to the activity of Ang II and endothelin (vasoconstrictors). The low NO activity might be a result of: 1) insufficient increment of the NO level in the vessels to compete with the increase in vasoconstrictor level and activity; and 2) inability of NO to act on its target site, as a consequence of death or injuries of its target cells (vascular smooth muscle cells). These hypotheses were investigated by administration of l-arginine and SNP prior to the induction of ischemia. The urinary excretion was increased following l-arginine and SNP adminis-

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**Fig. 7.** Light Micrographs of the Renal Cortex of the Non-ischemic (A) and Ischemic Kidneys (B) of the I/R Untreated Rat, Ischemic Kidney of the Rat with l-Arginine Pre-treatment (C), l-NAME Pre-treatment (D), SNP Pre-treatment (E), SNP Post-treatment (F), Losartan Treatment (G), Captopril Pre-treatment (H), Captopril Post-treatment (I), and BQ-123 Treatment (J)

G: Glomerulus, PCT: proximal convoluted tubule, DCT: distal convoluted tubule, and *: cell debris (H&E, ×400).
trations without significant changes in the plasma NO. Since GFR and RBF did not show any improvement over that of the untreated group, it is possible that NO may not exert its effect on the vascular smooth muscle. Another possible explanation is that the drugs were given so early that when the ischemia was induced the increased NO had been all metabolized and could no longer affect the GFR and RBF. In this study, the drugs were administered 40 min prior to the ischemic induction, whereas the half-life of NO is only 3–5 s. In order to solve the problem concerning the half-life of NO, SNP was given continuously to the rat after ischemic induction (during reperfusion period). Surprisingly, plasma NOx and the urinary excretion of NOx from both kidneys after SNP administration under this condition were not significantly increased from their own control values. The possible explanation for this observation is that the excess NO may be bound to sulf-hydryl (SH) groups present in proteins forming S-nitrosothiols (RS-NO). Therefore, no increments in both plasma NO and urinary NOx excretion were observed. The beneficial effect is thus expected to come from the "quenching" of large amounts of NO that prevent the generation of peroxynitrite (ONOO-). In this group, the degree of decreases in RBF and GFR were lessened.

An increased FeNa indicated an impairment of the tubular function following I/R injury. NO decreases tubular reabsorption of Na+ directly by inhibition of Na+-K+ ATPase activity and indirectly by induction of cellular injury via production of free radicals. In contrast, Ang II increases Na+ reabsorption by stimulating proximal tubule sodium transport. However, the relative activity of NO and Ang II in the renal tubule under I/R condition is not known. An increase in FeNa after I/R might be a result of the higher NO activity that could decrease tubular reabsorption of Na+ and decreased the activity of Na+-K+ ATPase. This is consistent with the results of urinary NOx and Na+-K+ ATPase activity. In all groups treated with NO donor, tubular injuries were observed histologically except in the SNP post-treated group. The administration of NO inhibitor, l-NAME, severely suppressed the tubular function as indicated by decreased activity of Na+-K+ ATPase. This is possibly because RBF was markedly reduced.

Hypoxia/anoxia causes the release of diffusible vasoconstrictor substances including Ang II and endothelin. Ang II produces vasoconstriction by a direct action on vascular smooth muscle via AT1 receptors and the intra-renal effects of Ang II and ET, which mediated their effects via the activation of AT1 and ETA receptors, respectively. Therefore, in order to balance the activities of vasoconstrictor and vasodilator mediators, a modulation of vasoconstrictor activity is recommended. Among all drugs used in this study, losartan and SNP given to the rats during reperfusion provide the best results in improving various renal function parameters and morphology after I/R. The information from this study may be useful for further studies on the prevention or improvement of I/R injury of the kidney and for the selection of effective drugs for clinical treatment.

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**Conflict of Interest** The authors declare no conflict of interest.

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