Opposite Effects of Pre- and Postischemic Treatments with Nitric Oxide Donor on Ischemia/Reperfusion-Induced Renal Injury

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ABSTRACT

We have demonstrated previously that preischemic treatment with FK409 [(±)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide], a spontaneous nitric oxide (NO) donor, improves ischemia/reperfusion-induced renal injury. However, there is conflicting information (renoprotective or cytotoxic) as to the contribution of NO to ischemic acute renal failure (ARF). In the present study, we investigated the effect of postischemic treatment with FK409 (1, 3, and 10 mg/kg i.v.) at 6 h after reperfusion on ischemic ARF, in comparison with the preisch- emic treatment effect. Ischemic ARF was induced by clamping of the left renal artery and vein for 45 min, followed by reperfusion, 2 weeks after contralateral nephrectomy. Renal function in ARF rats markedly decreased at 24 h after reperfusion. Histopathological examination of the kidney of ARF rats revealed severe renal damage. In contrast to the renoprotective effect by preischemic treatment, postischemic treatment with FK409 aggravated the ischemia/reperfusion-induced renal dysfunction and histological damage. Immunohistochemical analysis of renal sections obtained from ARF rats revealed positive staining for nitrotyrosine, a biomarker of peroxynitrite formation, in injured tubular cells, and more intense staining was observed in renal tissues from the animals that received postischemic treatment with FK409. On the other hand, the formation of nitrotyrosine, neutrophil infiltration into renal tissues, and renal superoxide production, all of which were enhanced in ARF rats, were efficiently attenuated by the preischemic treatment with FK409. These results demonstrate that, although preisch- emic treatment with an NO donor is renoprotective, postisch- emic treatment with the same agent aggravates the ischemia/ reperfusion-induced renal injury, probably through peroxynitrite overproduction.

There is accumulating evidence that, in the kidney, various renal pathological conditions, such as chronic renal failure with renal mass reduction, lipopolysaccharide- provoked renal dysfunction, and ischemic acute renal failure (ARF) (Ashab et al., 1995; Caramelo et al., 1996; Schwartz et al., 1997).

ARF is a common clinical complication with an uncertain outcome, ranging from complete restitution to high mortality (Kelly and Molitoris, 2000). Ischemia, followed by reperfusion, is one of the major causes of ARF (Thadhani et al., 1996). Various in vivo studies have indicated that NO biosynthesis and its action are closely related to the pathogenesis of ischemia/reperfusion-induced ARF (Conger et al., 1988, 1991; Chintala et al., 1993; Schramm et al., 1994; Pryor and Squadrito, 1995). Conger et al. (1988, 1991) demonstrated that decreased endothelium-dependent vasorelaxation and NO production were related to an impaired renal function observed after ischemia/reperfusion.

ABBREVIATIONS: NO, nitric oxide; ARF, acute renal failure; NOS, NO synthase; eNOS, endothelial NOS; iNOS, inducible NOS; FK409, [(±)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide; BUN, blood urea nitrogen; Pcr, plasma creatinine concentration; Uosm, urinary osmolality; FENa, fractional excretion of sodium; Ccr, creatinine clearance; UF, urine flow.
The NO precursor L-arginine has been reported to ameliorate postischemic ARF (Schramm et al., 1994). Furthermore, the inhibition of NO synthase (NOS) was seen to aggravate the postischemic ARF (Chintala et al., 1993), thereby suggesting the renoprotective role of endogenous NO in this disease. On the other hand, NO may be deleterious because of its reactivity with oxygen free radicals produced during reperfusion of the ischemic kidney to yield toxic products, such as peroxynitrites (Pryor and Squadrito, 1995). Thus, NO seems to have bidirectional effects on the pathogenesis of ischemia/reperfusion-induced ARF, as suggested previously (Goligorsky and Noiri, 1999).

NO is synthesized by different NOS isozymes, which have been cloned and characterized: endothelial NOS (eNOS), neuronal NOS, and inducible NOS (iNOS) (Knowles and Moncada, 1994). It has been demonstrated that ischemia/reperfusion-induced renal injury is efficiently attenuated by genetic deficiency or the pharmacological blockade of iNOS (Ling et al., 1999; Walker et al., 2000; Chatterjee et al., 2002). Although iNOS-derived NO predominantly elicits pathophysiological effects, eNOS-derived NO is believed to be responsible for maintaining physiological renal hemodynamics and renal functional parameters (Goligorsky and Noiri, 1999). Most recently, it has been observed that there is a marked impairment of renal function in eNOS-deficient mice subjected to 45-min ischemia/reperfusion, leading to a further deterioration of the disease condition compared with wild-type mice (Yamasawa et al., 2005). Although the role of NO in the pathogenesis of ischemic ARF appears controversial, one interesting investigation has found that an NO donor, sodium nitroprusside, prevents the neutrophil-mediated ischemic ARF associated with cytokine release and increased epithelial permeability (Linas et al., 1997). It is also found that preischemic treatment with FK409, a spontaneous NO releaser, exerted a remarkable protective effect against the ischemia/reperfusion-induced ARF (Matsumura et al., 1998). The biological actions of FK409, such as vasorelaxation, in isolated blood vessels can be attributed to spontaneous NO release after decomposition of the compound (Yamada et al., 1991; Kita et al., 1994b) and antianginal effects (Kita et al., 1994c). In addition, the antithrombotic effect of FK409 was more potent than those of organic nitrates such as isosorbide dinitrate, the effects being based on the potential of spontaneous NO release. Thus, this compound seems to be useful for the abolition of functional roles of NO in the pathogenesis of ischemic ARF. Although FK409 exerted a beneficial effect on preischemic treatment (Matsumura et al., 1998), it remains to be determined whether the agent can improve the ischemia/reperfusion-induced renal injury when given after the reperfusion. In general, because ARF cannot be predicted in many clinical cases, it is most worthwhile to know whether the postischemic treatment is sufficient at least enhances the recovery process, or is detrimental. Thus, we investigated the effect of postischemic treatment with FK409, and the findings were compared with those observed by the preischemic treatment.

Materials and Methods

Animals and Experimental Design. Male Sprague-Dawley rats (10 weeks of age, Japan SLC, Shizuoka, Japan) weighing 280 to 320 g were used. The animals were housed in a light-controlled room with a 12-h light/dark cycle and were allowed ad libitum access to food and water. Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Research Committee at Osaka University of Pharmaceutical Sciences. Two weeks before the experiment (8 weeks of age), the right kidney was removed through a small flank incision under pentobarbital anesthesia (50 mg/kg i.p.). After a 2-week recovery period, uninephrectomized rats were divided into seven groups: sham-operated control, preischemic treatment, postischemic treatment with FK409 (1 mg/kg i.v.), postischemic treatment with FK409 (3 mg/kg i.v.), postischemic treatment with FK409 (3 mg/kg i.v.) + ARF, preischemic treatment with FK409 (3 mg/kg i.v.) + ARF, and postischemic treatment with FK409 (10 mg/kg i.v.) in ARF. Three days before ischemic ARF, rats were anesthetized with pentobarbital (50 mg/kg i.p.), and were separated through a small flank incision. The left renal artery and vein were excluded with a nontraumatic clamp at 45 min before the end of the ischemic period, the clamp was released after 3 h of reperfusion period. FK409, or its vehicle (a mixture of 2.5% ethanol, 0.5% polyethylene glycol 400, and 67.5% saline), was administered postischemic treatment at 6 h after the reperfusion. Postischemic treatment, 1 ml/kg into the tail vein. In sham-operated control animals, the left kidney was removed identically, except for the clamping. The animals were exposed to 45-min ischemia and were housed in metabolic cages 24 h after reperfusion; 5-h urine samples were taken from the animals every 5 h, and blood samples were drawn from the thoracic aorta at the 6th of the one collection period. The plasma samples were taken, and blood samples were drawn from the thoracic aorta at the end of the urine collection period. The plasma was separated by centrifugation. These samples were used for measurements of renal functional parameters. The kidneys were excised and examined using a dissecting microscope. In separate experiments, animals were sacriﬁced at various time points after the start of reperfusion for evaluation of renal dysfunction and superoxide production.

Renal Functional Parameters. Blood urea nitrogen (BUN) and plasma creatinine concentration (Pcr) or urine were determined using enzymatic assay kit, the BUN-test-Wako, and Creatinine-test-Wako (Wako Pure Chemicals, Osaka, Japan), respectively. Urinary osmolality (Uosm) was measured by freezing point depression (Fiske Associates, Norwood, MA). Urine and plasma sodium concentrations were determined using an atomic absorption spectrophotometer (205D; Hitachi, Hitachiinaka, Japan). The fractional rate of sodium excretion (FENa; percentage) was calculated from the following formula: 

\[ \text{FENa} = \frac{\text{U Na} \times \text{P Na}}{\text{U osm}} \times 100 , \]

where U Na is the urinary sodium excretion, and P Na is the plasma sodium concentration.

Histological Studies. Excised left kidneys were processed for light microscopic observation, according to standard procedures. The kidneys were then fixed in phosphate-buffered 10% formalin, after which the kidneys were chopped into small pieces, embedded in paraffin wax, cut at 4 µm, and stained with H&E. Histopathological changes were analyzed for tubular necrosis, proteinaceous casts, and medullary congestion, as suggested by Solez et al. (1974). Tubular necrosis and proteinaceous casts were graded as follows: no damage (0), mild (1, unicellular, patchy isolated damage), moderate (2, damage less than 25%), severe (3, damage between 25 and 50%), and very severe (4, more than 50% damage). The degree of medullary congestion was defined as: no congestion (0), mild (1, vascular congestion with identification of erythrocytes by ×400 magnification), moderate (2, vascular congestion with identification of erythrocytes by ×200 magnification), severe (3, vascular congestion with identification of erythrocytes by ×100 magnification), and very severe (4, vascular congestion with identification of erythrocytes by ×40). The scoring of the histological data was performed by independent observers in a double-blind manner.

Measurement of Renal O₂ Production. Renal O₂ production was measured using a lucigenin-enhanced chemiluminescence assay.
(Skatchkov et al., 1999). The whole kidney was removed from rats and cut into strips (2-mm pieces). Immediately, renal tissue segments were placed in test tubes containing modified Krebs-HEPES buffer (pH 7.4, 99.01 mM NaCl, 4.69 mM KCl, 1.87 mM CaCl2, 1.20 mM MgSO4, 1.03 mM K2HPO4, 25 mM Na-HEPES, and 11.1 mM glucose) and allowed to equilibrate in the dark for 15 min at 37°C before measurement. After the equilibration, lucigenin (5 μM) was added to the tube, and then the luminescence was measured using a luminometer (Sirius-2; Berthold Technologies, Bad Wildbad, Germany). The relative light unit was integrated every 3 s for 15 min and averaged. The renal O2 production was expressed as relative light unit per minute per milligram dry tissue weight.

**Immunohistochemical Analysis.** Nitrotyrosine formation, a marker of peroxynitrite formation, in the kidney was determined using immunohistochemical staining. Paraffin-embedded tissue sections (4 μm) were cleared in xylene, ethanol, and washed in phosphate-buffered saline. Slides were incubated in methanol with 3% H2O2 for 20 min to block endogenous peroxidase activity. Nonspecific protein binding was blocked by incubation with 10% normal rabbit serum (Histofine SAB-PO kit; Nichirei, Tokyo, Japan). Mouse antinitrotyrosine antibody (Zymed Laboratories, South San Francisco, CA) was incubated with the sections for 1 h at room temperature. After incubation with primary antibody, specific labeling was detected using a biotin-conjugated rabbit anti-mouse immunoglobulin (Histofine SAB-PO kit) and then avidin-biotin conjugated peroxidase (Histofine SAB-PO kit; Nichirei). Samples were then viewed under a light microscope.

**Neutrophil Infiltration.** Neutrophil infiltration was evaluated using naphthol AS-D chloroacetate esterase staining (91C; Sigma-Aldrich, St. Louis, MO) (Moloney et al., 1960; Chiao et al., 1997) by counting the number of neutrophil present in the outer zone of the medulla of the kidneys. Neutrophils were counted in 50 randomly selected high-power fields (×400) of the outer zone of the medulla. Data were expressed as neutrophils per millimeter squared of tissue.

**Drug.** FK409, a kind gift from Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan), was dissolved in a mixture of 2.5% ethanol, 30% polyethylene glycol 400, and 67.5% saline immediately prior to administration.

**Statistical Analysis.** Values are expressed as the mean ± S.E.M. Relevant data were assessed by ANOVA, followed by Dunnett’s tests for multiple comparisons. For all comparisons, differences were considered significant at P < 0.05.

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**Results**

**Time Course of Blood Urea Nitrogen after the Ischemia/Reperfusion.** First, we examined the time course profile of BUN in the ARF rats. As shown in Fig. 1, BUN levels in rats subjected to 45-min ischemia increased gradually after the reperfusion. At 2 h after the reperfusion, there was an apparent increase at 6 h after the reperfusion. Thereafter, the BUN levels were markedly elevated. Therefore, in the following experiments to evaluate whether the postischemic treatment with FK409 is beneficial or detrimental on the ischemic ARF, this agent was administered at 6 h after the start of reperfusion.

**Renal Function after the Ischemia/Reperfusion and Effects of Postischemic Treatment with FK409.** The renal function of rats subjected to 45-min ischemia/reperfusion.

**Histological Renal Damage after the Ischemia/Reperfusion and Effects of Postischemic Treatment with FK409.** Histopathological examinations revealed severe lesions in the kidney of vehicle-treated ARF rats (24 h after the ischemia/reperfusion). These changes were characterized by tubular necrosis in the outer zone outer stripe of the medulla (Fig. 3B) (scores, 3.17 ± 0.17; Fig. 4), medullary congestion, and hemorrhage in the outer zone inner stripe of the medulla (Fig. 3F) (scores, 2.83 ± 0.17; Fig. 4) and proteinaceous casts in tubuli in the inner zone of the medulla (Fig. 3J) (scores, 2.83 ± 0.17; Fig. 4). Postischemic treatment with FK409 worsened the development of all these lesions in a dose-related manner (Figs. 3 and 4).

**Time Course of Renal O2 Production after the Ischemia/Reperfusion and Effects of Postischemic Treatment with FK409.** As shown in Fig. 5, renal O2 production in rats subjected to 45-min ischemia increased gradually after the reperfusion. At 6 h after the reperfusion, there was a significant and marked increase in the renal O2 level; thereafter, its level reached a plateau dur-
ing the first 24 h after the reperfusion. When 10 mg/kg FK409 was given at 6 h after the reperfusion, increased renal $O_2^-$ production was temporarily and markedly reduced from 6.5 to 8 h after the reperfusion, i.e., from 0.5 to 2 h after the administration of FK409, but the reduced level of renal $O_2^-$ production was restored between 12 and 24 h after the reperfusion. Similar decreases in renal $O_2^-$ production were observed even when a lower dose of FK409 (3 mg/kg) was given (data not shown).

**Nitrotyrosine Formation in the Kidney after the Ischemia/Reperfusion and Effects of Pre- or Post-ischemic Treatment with FK409.**

Tyrosine nitration has been used as an index of the nitrosylation of protein by peroxynitrite (Walker et al., 2000). Compared with renal sections of sham-operated rats, immunohistochemical analysis of renal sections obtained from vehicle-treated ARF rats at 24 h after the ischemia/reperfusion revealed positive staining for nitrotyrosine in tubules (Fig. 6, C and D). Furthermore, more intense staining was observed in renal sections obtained from ARF rats given postischemic FK409 treatment (10 mg/kg) (Fig. 6, E and F). Similar nitrotyrosine-positive staining was increased by the postischemic treatment at the lower dose (3 mg/kg) (Fig. 7A). On the other hand, when the same dose of FK409 was administered at 5 min prior to the ischemia, reduced nitrotyrosine staining was observed (Fig. 7B) compared with the case of the vehicle-treated ARF rats. These findings suggest that although the postischemic FK409 treatment augments the peroxynitrite formation in the kidney subjected to the ischemia/reperfusion, the preischemic treatment with the same agent suppresses it.

**Neutrophil Infiltration in the Kidney after the Ischemia/Reperfusion and Effects of Pre- or Post-ischemic Treatment with FK409.**

We evaluated...
whether preischemic treatment with FK409 suppressed the neutrophil infiltration into renal tissue, an event that has been known to produce O$_2^-$ (Clancy et al., 1992) and is believed to be one of the main causal factors of the ischemia/reperfusion-induced ARF (Linas et al., 1992). As shown in Fig. 8B, neutrophils were observed in the kidney of vehicle-treated ARF rats 6 h after the reperfusion. The number of infiltrating neutrophils in the vehicle-treated ARF rats was significantly increased compared with that in the sham-operated rats (Figs. 8A and 9A). On the other hand, the neutrophil infiltration was markedly suppressed in the renal tissues of ARF rats given FK409 (3 mg/kg) prior to the 45-min ischemia (Figs. 8C and 9A). When the neutrophil infiltration was determined 24 h after the reperfusion, there was a slight but significant increase, which tended to be suppressed by the preischemic FK409 treatment (Figs. 8D and E, and 9B), but not by the postischemic treatment.

**Effects of Preischemic Treatment with FK409 on Renal O$_2^-$ Production after the Ischemia/Reperfusion**

Finally, the effect of preischemic treatment with FK409 on renal O$_2^-$ production in ARF rats. As shown in Fig. 9C, the increased level of renal O$_2^-$ production at 6 h after the ischemia/reperfusion was markedly suppressed by treatment with FK409 (3 mg/kg) prior to the 45-min ischemia. Similar suppressive effects of the FK409 pre-treatment were observed at 24 h after the ischemia/reperfusion (Fig. 9D).

**Discussion**

Ischemic ARF is a frequent clinical syndrome with a high morbidity and mortality (Thadhani et al., 1996). Reperfusion of previously ischemic renal tissue initiates a series of complex cellular events that results in injury and the eventual death of renal cells due to a combination of apoptosis and necrosis (Lieberthal and Levine, 1996). The molecular mechanisms underlying the ischemia/reperfusion-induced renal injury are poorly understood, but it has been reported that several causal factors (ATP depletion, reactive oxygen species, phospholipase activation, neutrophil infiltration, vasoactive peptides, etc.) are contributive to the pathogenesis of this renal damage (Edelstein et al., 1997). We found that the postischemic treatment with
FK409 worsened the ischemia/reperfusion-induced renal dysfunction and related tissue injury, in contrast to our previous findings (Matsumura et al., 1998), indicating that FK409 administration prior to the ischemia markedly attenuated the ischemia/reperfusion-induced ARF. Thus, under the same experimental conditions using the same agent and animal species, we demonstrated the duality of the actions of NO in ischemia/reperfusion-induced ARF. One can speculate that renal and/or systemic hemodynamic effects of FK409 given before or after the ischemia may influence the postischemic renal function. In our previous study (Matsumura et al., 1998), the pretreatment with FK409 (1 mg/kg i.v.) failed to attenuate the immediate renal hemodynamic changes after the ischemia/reperfusion. In addition, we noted that the blood pressure-lowering effects of FK409 given before or after the ischemia were similar. Moreover, an i.v. administration of hydralazine, at the same hypotensive dose as FK409, had no effect on the ischemia/reperfusion-induced renal dysfunction (A. Nakajima, M. Takaoka, and Y. Matsumura, unpublished data). Thus, it is reasonable to consider that the contrasting effects obtained by the pre- and postischemic treatments with FK409 are independent of the drug-induced transient renal and/or systemic hemodynamic changes.

Several studies have demonstrated that endogenous or exogenous NO protects the kidney against ischemia/reperfusion injury (Chintala et al., 1993; Schramm et al., 1994; Linas et al., 1997). Most recently, we noted that both exogenous and endogenous NO have protective effects

Fig. 7. Immunohistochemical staining for nitrotyrosine in the kidney of ARF rats given post- (A) or pre- (B) ischemic treatment with 3 mg/kg FK409 24 h after the ischemia/reperfusion. FK409 was given i.v. 5 min before the ischemia or 6 h after the reperfusion. Arrows indicate nitrotyrosine (magnification, ×200).
against ischemia/reperfusion-induced renal dysfunction and tissue injury, at least in part, through the suppression of endothelin-1 production (Kurata et al., 2004). The overproduction of this peptide in the postischemic kidney is known to be one of the major causal factors of this disease (Wilhelm et al., 1999; Matsumura et al., 2000). In addition, we noted that preischemic treatment with FK409 suppressed the enhancement of renal sympathetic nerve activity in the postischemic kidney (H. Kurata, M. Takaoka, and Y. Matsumura, unpublished data), which is closely related to the renal dysfunction in the postischemic kidney (Fujii et al., 2003). The present study clearly demonstrated that the preischemic treatment with FK409 markedly suppressed the renal O$_2^-$ production augmented by the ischemia/reperfusion, following the attenuation of neutrophil infiltration/migration (Fig. 9). Neutrophil infiltration/migration has been shown to contribute to the postischemic ARF through various mechanisms (Linas et al., 1988, 1992) noted that mild renal ischemia and primed neutrophils synergistically enhanced renal ischemic injury. In addition, monoclonal antibodies to neutrophil adhesion molecules are known to decrease the postischemic renal injury (Dai et al., 1994). Kubes et al. (1991) found that neutrophil adhesion in postcapillary venules was markedly enhanced by an NO synthase inhibitor and that the inhibitor reversed the enhancement was prevented by L-arginine, thereby suggesting that NO may be an important endogenous inhibitor of neutrophil adhesion in venules. Moreover, NO is reported to inhibit neutrophil O$_2^-$ production via direct effects on the membrane components of the NADPH oxidase (Clancy et al., 1992). Taken together, FK409, given prior to the ischemia, exerted a renoprotective effect via multifunctional mechanisms.

The postischemic treatment with FK409 was performed 6 h after the start of reperfusion because the ischemia/reperfusion-induced renal dysfunction was apparent at the same time. Moreover, the enhancement of renal O$_2^-$ production was also observed. FK409 administration temporarily decreased the renal O$_2^-$ level and was followed by intense positive nitrotyrosine staining, suggesting the augmentation of peroxynitrite formation. The ischemia/reperfusion-induced renal dysfunction and tissue injury were much more severe in animals given the postischemic treatment with FK409. Such a deteriorating effect of FK409 was not observed when the agent was given immediately after the start of reperfusion (data not shown), and then renal O$_2^-$ production did not increase. The rate constant for the reaction of O$_2^-$ with NO is known to be 3-fold higher than that with superoxide dismutase (Crow and Beckman, 1996). Thus, it is most likely that FK409-derived NO reacts with O$_2^-$ to form peroxynitrite, which causes injury via direct oxidant injury and protein tyrosine nitration (Beckman, 1996; Radi et al., 2001).

Nitrotyrosine formation has been detected in several oxidant-mediated disease models, such as myocardial ischemia/reperfusion injury (Liu et al., 1997), acute pulmonary inflammation (Kooy et al., 1997), and lipopolysaccharide-induced renal injury (Zhang et al., 2000). In ischemia/reperfusion-induced ARF models, nitrotyrosine-protein adducts are observed in the tubular epithelium approximately 6 to 24 h after the ischemia/reperfusion (Chiao et al., 1997; Walker et al., 2000; Chatterjee et al., 2002). Recent studies (Walker et al., 2000; Chatterjee et al., 2002, 2003) demonstrated that iNOS inhibitors could improve the ischemia/reperfusion-induced renal dysfunction and tissue injury and reduce nitrotyrosine formation, suggesting that iNOS-generated NO mediates the above renal...
damage through peroxynitrite formation. Most recently, Schneider et al. (2003) demonstrated that iNOS expression in the postischemic kidney (24 h after the ischemia/reper-
fusion) markedly increased to 4-fold of the control, in con-
trast to the moderate down-regulation of eNOS expression. Taken together, iNOS-derived NO seems to contribute to the pathophysiology of ischemia/reperfusion-induced ARF, as suggested (Goligorsky and Noiri, 1999). Selective iNOS inhibitors may be useful against renal dysfunction and injury induced by ischemia/reperfusion of the kidney in humans. Furthermore, this view is strongly supported by the evidence that kidneys of iNOS knockout mice are pro-
tected against ischemia/reperfusion-induced ARF (Ling et
al., 1999; Chatterjee et al., 2003). Thus, the aggravation of ischemia/reperfusion-induced ARF by postischemic treatment with FK409 seems to reflect exaggerated responses to the pathological effect of iNOS-derived NO.

In the present study, FK409 given after reperfusion aggravated the ischemia/reperfusion-induced renal dys-
function and tissue injury, and these lesions were accom-
panied by enhanced nitrotyrosine formation in the tubular epithelium, suggesting that the increment of peroxynitrite formation is closely related to the above lesions. In con-
trast, the preischemic administration of FK409 signif-
dically reduced neutrophil infiltration, renal O2


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