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Protective effect of nitric oxide on ischemia/reperfusion-induced renal injury and endothelin-1 overproduction

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Abstract

To elucidate the role of nitric oxide (NO) in the pathogenesis of ischemic acute renal failure, we examined the effects of (±)-(*E*)-4-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexenamide (FK409) and N^G-nitro-L-arginine methyl ester (L-NAME) as a NO donor and a non-selective NO synthase inhibitor on ischemia/reperfusion-induced renal injury and renal endothelin-1 content. Ischemic acute renal failure was induced by occlusion of the left renal artery and vein for 45 min followed by reperfusion, 2 weeks after contralateral nephrectomy. At 24 h after reperfusion, renal function in untreated acute renal failure rats markedly decreased and histological examination revealed severe renal damage. In addition, increases in renal endothelin-1 contents were evident in the acute renal failure rats at 2, 6, and 24 h after reperfusion, respectively. Pretreatment with FK409 (1 or 3 mg/kg, i.v.) attenuated ischemia/reperfusion-induced renal dysfunction, histological damage, and endothelin-1 overproduction after reperfusion. In contrast, pretreatment with L-NAME (1 or 10 mg/kg, i.v.) aggravated renal injuries of acute renal failure rats at 24 h after reperfusion, and the effect is accompanied by further increases in the renal endothelin-1 content at 2 and 6 h, but not at 24 h, after reperfusion. These results suggest that suppressive effects of NO on the renal endothelin-1 overproduction induced by ischemia/reperfusion in an early phase are probably responsible for the protective effect of NO against ischemic acute renal failure.

Keywords: Nitric oxide; Acute renal failure; Ischemia/reperfusion; Endothelin-1

1. Introduction

Endothelin-1 is a potent vasoconstrictor peptide isolated from the culture supernatant of porcine aortic endothelial cells (Yanagisawa et al., 1988). This peptide participates in hypertension, vasospasm, atherosclerosis, and ischemia/reperfusion injury (Schiffrin, 1995). In particular, there is growing evidence that endothelin-1 is involved in the development of ischemic acute renal failure. The kidney is well known for synthesizing endothelin-1 and expressing both endothelin ET_A and ET_B receptors (Nambi et al., 1992). Furthermore, it has been demonstrated that endothelin-1 content (Shibouta et al., 1990; Kuro et al., 2000) and endothelin-1 mRNA expression (Firth and Ratcliffe, 1992; Wilhelm et al., 1999) are elevated in postischemic kidneys. In addition to these circumstantial evidence, both endothelin ET_A-selective and nonselective ET_A/ET_B receptor antagonists have been reported to attenuate the ischemia/reperfusion-induced impairment of renal function (Mino et al., 1992; Gellai et al., 1995; Birck et al., 1998; Kuro et al., 2000). Taken together, it is conceivable that up-regulated renal endothelin-1 by ischemia/reperfusion is involved in the pathogenesis of ischemic acute renal failure exclusively via endothelin ET_A receptor activation.

It is well known that nitric oxide (NO) can reduce endothelin-1 production in endothelial cells (Boulanger and Lüscher, 1991; Mitsutomi et al., 1999). Mitsutomi et al. (1999) examined various spontaneous NO donors and NO synthase inhibitors on endothelin-1 production in porcine cultured endothelial cells, and found that NO donors

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inhibited endothelin-1 production, whereas NO synthase inhibitors increased endothelin-1 production.

In the kidney, NO is synthesized and plays an important role in regulating renal hemodynamics and function (Ruilope et al., 1994). 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 (Shultz et al., 1991), macula densa (Mundel et al., 1992), or tubular cells (Terada et al., 1992), thereby suggesting that endogenous NO plays an important role in the regulation of renal hemodynamics and functions. The role of NO in ischemia/reperfusion-induced acute renal failure is still controversial. Yu et al. (1994) reported that N^G-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor, prevented hypoxia/reoxygenation injury in isolated rat proximal tubules. They also found, in this in vitro model, that sodium nitroprusside, a NO donor, or L-arginine, the NO precursor, enhanced the injury. These results suggest that NO is synthesized in proximal tubules and participates in tubular hypoxia/reoxygenation injury as one of the mediators. In contrast, in vivo studies have shown a beneficial effect of NO in ischemic acute renal failure models. Indeed, Chintala et al. (1993) reported that the inhibition of NO production with N^G-monomethyl-L-arginine (L-NMMA), a non-selective NO synthase inhibitor, significantly deteriorated renal function of the postischemic kidney in anesthetized rats, whereas pretreatment with L-arginine abolished the NO synthase inhibitor-induced renal dysfunction. We have also noted that renal dysfunction in ischemic acute renal failure rats is markedly attenuated by treatment with (±)-(*E*)-4-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexenamide (FK409), a spontaneous NO donor (Matsumura et al., 1998). Taken together, NO seems to have a protective effect against renal injury at least in in vivo models of ischemic acute renal failure; however, there are no data indicating that endogenous and exogenous NO can suppress the ischemia/reperfusion-induced renal overproduction of endothelin-1, a deleterious mediator in the pathogenesis of ischemic acute renal failure.

The purpose of the present study is to evaluate the effects of endogenous and exogenous NO on ischemia/reperfusion-induced renal dysfunction and histological damage, on the same experimental conditions. To attain the objective, we used FK409 as a NO donor and L-NAME as a NO synthase inhibitor, respectively, and we investigated whether the effects of FK409 and L-NAME on the ischemic acute renal failure would be associated with ischemia/reperfusion-induced renal endothelin-1 overproduction.

2. Materials and methods

2.1. Animal and experimental design

The experimental protocol is identical with that in our previous report showing that FK409 has protective effects on ischemic acute renal failure in rats (Matsumura et al., 1998). Male Sprague–Dawley rats (280–300 g, 10 weeks of age; Japan SLC, Inc., Shizuoka, Japan) 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 Committee at Osaka University of Pharmaceutical Sciences (Osaka, Japan). Two weeks before the study (at 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, these rats were separated into six groups: (1) sham-operated control; (2) untreated ischemic acute renal failure; (3) ischemic acute renal failure pretreated with FK409 (1 mg/kg, i.v.); (4) ischemic acute renal failure pretreated with FK409 (3 mg/kg, i.v.); (5) ischemic acute renal failure pretreated with L-NAME (1 mg/kg, i.v.); and (6) ischemic acute renal failure pretreated with L-NAME (10 mg/kg, i.v.). To induce ischemic acute renal failure, the rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and the left kidney was exposed through a small flank incision. The left renal artery and vein were occluded with a nontraumatic clamp for 45 min. At the end of the ischemic period, the clamp was released to allow reperfusion. FK409, L-NAME, or vehicle (0.9% saline) in a volume of 1 ml/kg was injected intravenously, 5 min before the occlusion. In sham-operated control rats, the kidney was treated identically, except for the clamping.

Animals exposed to 45-min ischemia were housed in metabolic cages 24 h after the ischemia. At the end of urine collection for 5 h, blood samples were drawn from the thoracic aorta, and then the left kidneys were excised under pentobarbital anesthesia (50 mg/kg, i.p.). The plasma was separated by centrifugation. These samples were used for measurement of renal function parameters.

In separate experiments, left kidneys were obtained from animals at 2 and 6 h after reperfusion and used for endothelin-1 measurement.

2.2. Analytical procedures

Blood urea nitrogen and creatinine levels in plasma or urine were determined using the blood urea nitrogen test (Wako) and creatinine test (Wako, Osaka, Japan), respectively. Creatinine clearance (C_{cr}; ml/min/kg) was calculated from the formula $C_{cr} = U_{cr} \times UF / P_{cr}$, where U_{cr} and P_{cr} are creatinine concentration in urine and plasma, and UF is urine flow. Urinary osmolality (U_{osm}) was measured by freezing-point depression (Fiske Associates, Norwood, MA, USA). Urine and plasma sodium concentrations were determined using a flame photometer (205D; Hitachi, Ibaraki, Japan). Fractional excretion of sodium (FE_{Na}, %) was calculated from the formula $FE_{Na} = U_{Na}V / (P_{Na} \times C_{cr}) \times 100$, where $U_{Na}V$ is urinary excretion of sodium and P_{Na} is the plasma sodium concentration.

2.3. Histological studies

The kidneys were preserved in phosphate-buffered 10% formalin, after which the kidneys were chopped into small pieces, embedded in paraffin wax, cut at 3 μm, and stained with hematoxylin and eosin. Histopathological changes were analyzed for tubular necrosis, proteinaceous casts, and medullary congestion and hemorrhage, as suggested by Solez et al. (1974). Tissue injuries were graded as follows: no change (− or 0), mild (± or 1; unicellular, patchy isolated damage), moderate (+ or 2; damage less than 25%), severe (++ or 3; damage between 25% and 50%), and

very severe (+++ or 4; more than 50% damage). The evaluations were made by an observer who was blind to the treatment origin of the tissue.

2.4. Renal endothelin-1 assay

Endothelin-1 was extracted from the kidney, according to the method described elsewhere (Fujita et al., 1995). Briefly, kidneys were weighed and homogenized for 60 s in 8 vol of ice-cold organic solution (chloroform/methanol, 2:1, including 1 mM *N*-ethylmaleimide). The homogenates were left overnight at 4 °C and then 0.4 vol of distilled water was added, after which the homogenates were centrifuged at 1500×*g* for 30 min and the supernatant was stored. Aliquots of the supernatant were diluted 1:10 with a 0.09% trifluoroacetic acid solution and applied to Sep-Pak C_{18} cartridges. The sample was eluted with 3 ml of 63.3% acetonitrile and 0.1% trifluoroacetic acid. Eluates were dried in a centrifugal concentrator, and the dried residue was reconstituted in an assay buffer for radioimmunoassay. The clear solution was subjected to radioimmunoassay. Recoveries of endothelin-1 from renal tissue by this extraction procedure are approximately 80%. Radioimmunoassay for endothelin-1 was done, as described elsewhere (Matsumura et al., 1990), using endothelin-1 antiserum (a generous gift from Dr. Marvin R. Brown, Department of Medicine, University of California, San Diego, CA) that does not cross-react with big endothelin-1.

2.5. Drugs

FK409 was provided by Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan), and L-NAME was purchased from Wako. As the half-life of FK409 (46 min) is short (Kato et al., 1996), the drug was dissolved in saline (0.9%) just before administration. L-NAME was treated in the same manner. Other chemicals were obtained from Nacalai Tesque (Kyoto, Japan) and Wako.

2.6. Statistical analysis

Values were expressed as mean±S.E.M. The data were analyzed for significant differences between the sham-operated and untreated acute renal failure groups using Student's unpaired *t* test. Statistical analysis for renal functional studies was performed using one-way analysis of variance followed by a Dunnett-type multiple comparison tests. Histological data were analyzed using Kruskal–Wallis nonparametric test combined with a Steel-type multiple comparison test. For all comparisons, differences were considered significant at $P<0.05$.

3. Results

3.1. Renal function after the ischemia/reperfusion and effects of FK409 or L-NAME

As shown in Fig. 1, renal function of rats subjected to a 45-min ischemia showed a marked deterioration when measured at 24 h after reperfusion. As compared with sham-operated rats, untreated acute renal failure rats showed significant increases in blood urea nitrogen (24.3±1.3 versus 95.4±3.4 mg/dl), plasma creatinine concentration (0.82±0.07 versus 2.84±0.50 mg/dl) and fractional excretion of sodium (0.38±0.07% versus 2.10±0.52%), and significant decreases in creatinine clearance (3.76±0.41 versus 1.20±0.22 ml/min/kg) and urinary osmolality (1550±117 versus 423±68 mOsm/kg). The administration of FK409 (1 mg/kg, 3 mg/kg, i.v.) to acute renal failure rats dose-dependently attenuated the ischemia/reperfusion-induced renal dysfunction. When FK409 was given at the higher dose, all renal function changes induced by the ischemia/reperfusion were significantly and markedly suppressed (blood urea nitrogen, 34.2±3.2 mg/dl; plasma creatinine concentration, 0.89±0.06 mg/dl; creatinine clearance, 3.22±0.36 ml/min/kg; urinary osmolality, 900±81 mOsm/kg; fractional excretion of sodium, 0.36±0.05%). In contrast, the administration of L-NAME (1 mg/kg, 10 mg/kg, i.v.) to acute renal failure rats dose-dependently aggravated the renal dysfunction in postischemic kidneys (Fig. 2). When L-NAME was given at the higher dose, renal function changes induced by the ischemia/reperfusion were significantly worsened (blood urea nitrogen, 204.7±4.6 mg/dl; plasma creatinine concentration, 4.69±0.19 mg/dl; creatinine clearance, 0.06±0.02 ml/min/kg; fractional excretion of sodium, 24.21±5.9%) except for urinary osmolality (295±25 mOsm/kg).

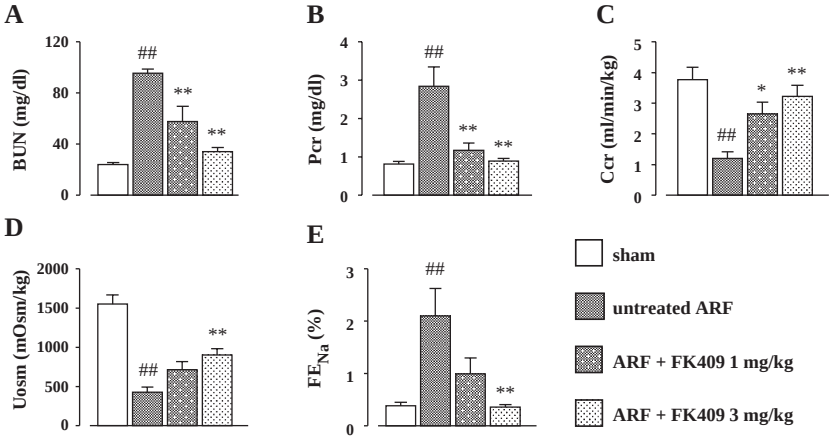

Fig. 1. Effect of FK409 on blood urea nitrogen (BUN; A), plasma creatinine concentration (P_{cr}; B), creatinine clearance (C_{cr}, C), urinary osmolality(U_{osm}; D), and fractional excretion of sodium (FE_{Na}; E) at 24 h after reperfusion. FK409 was given intravenously 5 min before ischemia. Each column and bar represents the mean±S.E.M. ($n=6$). $^{\#\#}P<0.01$, compared with sham-operated rats. $^{*}P<0.05$, $^{**}P<0.01$, compared with untreated acute renal failure rats. ARF, acute renal failure.

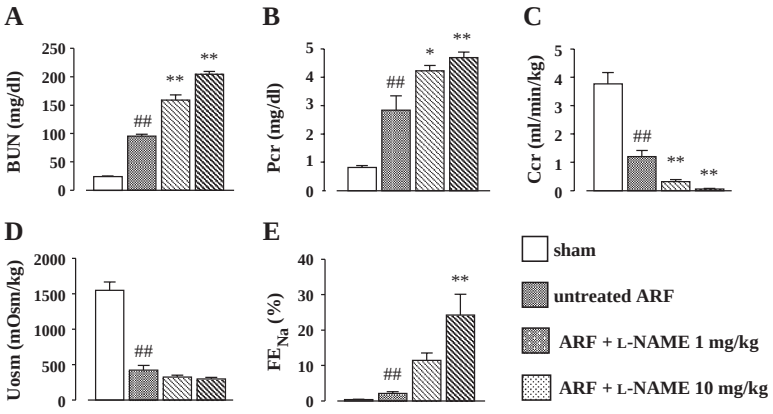

Fig. 2. Effect of L-NAME on blood urea nitrogen (BUN; A), plasma creatinine concentration (P_{cr}; B), creatinine clearance (C_{cr}; C), urinary osmolality(U_{osm}; D), and fractional excretion of sodium (FE_{Na}; E) at 24 h after reperfusion. L-NAME was given intravenously 5 min before ischemia. Each column and bar represents the mean ± S.E.M. ($n = 6$). $^{\#\#}P < 0.01$, compared with sham-operated rats. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with untreated ARF rats. ARF, acute renal failure.

3.2. Histological renal damage after the ischemia/reperfusion and effects of FK409 or L-NAME

Histopathological examination revealed severe lesions in the kidneys of untreated acute renal failure rats at 24 h after reperfusion. These changes were characterized by proteinaceous casts in tubuli in the inner zone of medulla (Fig. 3B), medullary congestion and hemorrhage in the outer zone inner stripe of medulla (Fig. 4B), and tubular necrosis in the outer zone outer stripe of medulla (Fig. 5B). Pretreatment with FK409 at the higher dose (3 mg/kg) attenuated the development of all lesions (Figs. 3C–5C). As shown in Table 1, FK409 dose-dependently and significantly prevented the tissue injuries. In contrast, pretreatment with L-NAME at the higher dose (10 mg/kg) showed more severe histopathological injuries (Figs. 3D–5D) than those seen in untreated acute renal failure rats. L-NAME tended to dose-dependently deteriorate the ischemia/reperfusion-induced tissue damage, although observed changes were not statistically significant (Table 1).

3.3. Renal endothelin-1 content after the ischemia/reperfusion and effects of FK409 or L-NAME

As shown in Table 2, renal endothelin-1 contents at 24 h after reperfusion were significantly increased in untreated acute renal failure rats, being about twofold over the sham-operated group. Pretreatment with FK409 dose-dependently suppressed the ischemia/reperfusion-induced increases in renal endothelin-1 contents. On the other hand, pretreatment with L-NAME unexpectedly did

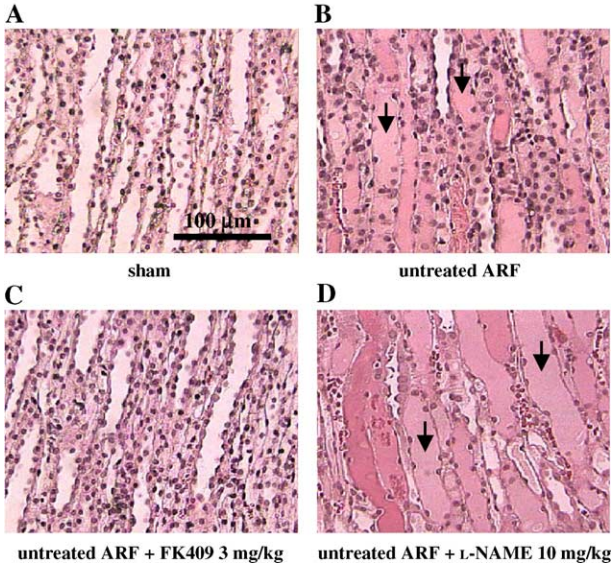

Fig. 3. Light microscopy of the inner zone of medulla of the kidney of ARF rats untreated (B) and treated with FK409 (3 mg/kg; C) or L-NAME (10 mg/kg; D) at 24 h after reperfusion, and sham-operated rats (A). FK409 or L-NAME was given intravenously 5 min before ischemia. Arrows indicate proteinaceous casts in tubuli (hematoxylin and eosin staining). ARF, acute renal failure.

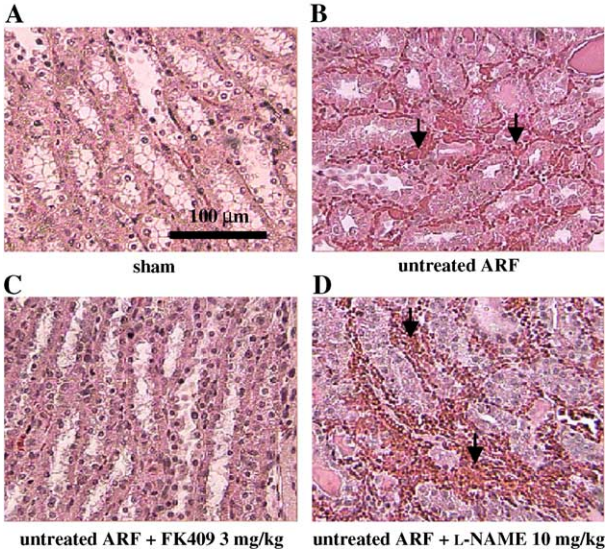

Fig. 4. Light microscopy of the outer zone inner stripe of medulla of the kidney of acute renal failure rats untreated (B) and treated with FK409 (3 mg/kg; C) or L-NAME (10 mg/kg; D) at 24 h after reperfusion, and sham-operated rats (A). FK409 or L-NAME was given intravenously 5 min before ischemia. Arrows indicate congestion and hemorrhage (hematoxylin and eosin staining). ARF, acute renal failure.

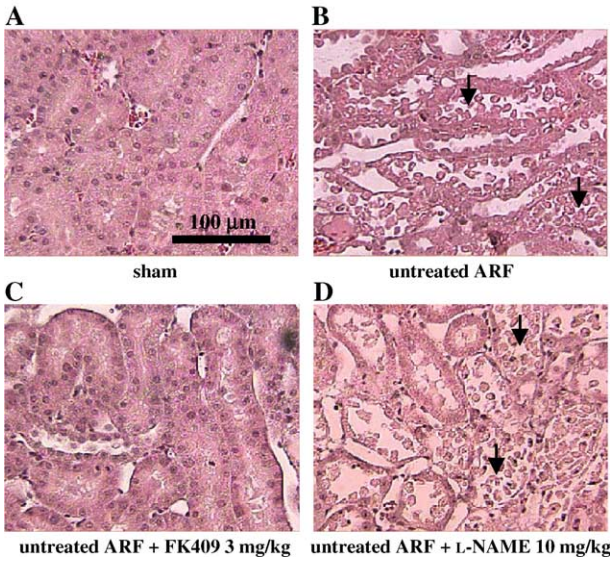

Fig. 5. Light microscopy of the outer zone outer stripe of medulla of the kidney of acute renal failure rats untreated (B) and treated with FK409 (3 mg/kg; C) or L-NAME (10 mg/kg; D) at 24 h after reperfusion, and sham-operated rats (A). FK409 or L-NAME was given intravenously 5 min before ischemia. Arrows indicate tubular necrosis (hematoxylin and eosin staining). ARF, acute renal failure.

not affect the increased endothelin-1 contents at 24 h after reperfusion, even at the higher dose. We next measured the renal endothelin-1 contents at 2 and 6 h after reperfusion (Fig. 6), based on recent observations that endothelin-1 overproduction occurs at an early phase after the ischemia/reperfusion of the kidney (Wilhelm et al., 1999; Kuro et al., 2000). Renal endothelin-1 contents in untreated acute renal failure rats increased at 2 h after reperfusion (0.49±0.04 ng/g tissue), compared with those seen in sham-operated rats (0.23±0.02 ng/g tissue). This increment was more marked at 6 h after reperfusion (0.70±0.03 ng/g tissue). Pretreatment with FK409 at 3 mg/kg significantly suppressed the elevation of renal endothelin-1 contents at these early phases (0.33±0.02 and 0.33±0.04 ng/g tissue, at 2 and 6 h, respectively). In contrast, pretreatment with L-NAME at 10 mg/kg markedly enhanced the renal endothelin-1 contents at 2 and 6 h after reperfusion (0.70±0.04 and 1.04±0.10 ng/g tissue, at 2 and 6 h, respectively), differently from the results obtained at 24 after reperfusion.

Table 2
Effect of FK409 or L-NAME on renal endothelin-1 contents at 24 h after reperfusion

Experimental group	Renal endothelin-1 content (ng/g tissue)
Sham ($n=6$)	0.23±0.02
Untreated ARF ($n=6$)	0.46±0.03*
ARF+FK409 1 mg/kg ($n=6$)	0.29±0.05**
ARF+FK409 3 mg/kg ($n=6$)	0.23±0.04**
ARF+L-NAME 1 mg/kg ($n=6$)	0.44±0.05
ARF+L-NAME 10 mg/kg ($n=6$)	0.47±0.06

FK409 or L-NAME was given intravenously 5 min before ischemia.
ARF, acute renal failure.
* $P<0.01$, compared with sham-operated rats.
** $P<0.01$, compared with untreated ARF rats.

4. Discussion

FK409 is a structurally unique vasodilator discovered from the fermentation product of *Streptomyces griseosporeus* (Hino et al., 1989). Kita et al. (1994a) reported that biological actions of FK409 can be accounted for by spontaneous NO release after decomposition of the compound. FK409 produces a potent vasorelaxation in the rat aorta (Isono et al., 1993). Furthermore, it has been noted that antiplatelet effects (Kita et al., 1994b) and antianginal effects (Kita et al., 1994c) are more potent than those of organic nitrates such as isosorbide dinitrate—these effects being based on the potential of spontaneous NO generation. It is also reported that FK409 is a short-lived substance with the half-life of 46 min (Kato et al., 1996). In our previous experiments (Matsumura et al., 1998), the levels of NO_x ($NO_2^-+NO_3^-$) in renal venous plasma of anesthetized rats, immediately after the ischemia for 45 min and reperfusion,

Table 1
Effect of FK409 or L-NAME on histopathological changes at 24 h after reperfusion

	Medullary congestion					Proteinaceous casts in tubuli					Tubular necrosis				
Histopathological	–	±	+	++	+++	–	±	+	++	+++	–	±	+	++	+++
Changes/grade	(0	1	2	3	4)	(0	1	2	3	4)	(0	1	2	3	4)
Untreated ARF ($n=6$)	0	0	0	3	3	0	0	1	3	2	0	0	1	3	2
	(3.50±0.22)					(3.17±0.31)					(3.17±0.31)				
ARF+FK409 1 mg/kg ($n=6$)	0	3	3	0	0	1	3	2	0	0	0	3	3	0	0
	(1.17±0.35)*					(1.17±0.31)*					(1.50±0.22)*				
ARF+FK409 3 mg/kg ($n=6$)	3	3	0	0	0	4	2	0	0	0	4	1	1	0	0
	(0.50±0.22)*					(0.33±0.21)*					(0.50±0.34)*				
ARF+L-NAME 1 mg/kg ($n=6$)	0	0	1	2	3	0	0	0	3	3	0	0	0	2	4
	(3.33±0.33)					(3.50±0.22)					(3.67±0.21)				
ARF+L-NAME 10 mg/kg ($n=6$)	0	0	1	1	4	0	0	0	2	4	0	0	0	1	5
	(3.50±0.34)					(3.67±0.21)					(3.83±0.17)				

Data are expressed as the number of animals with histopathological changes. Values in parentheses represent the mean±S.E.M. of histopathological change/grade. Grades: no change (–, 0), mild (±, 1), moderate (+, 2), severe (++, 3), very severe (+++, 4).
ARF, acute renal failure.
* $P<0.01$, compared with untreated ARF rats.

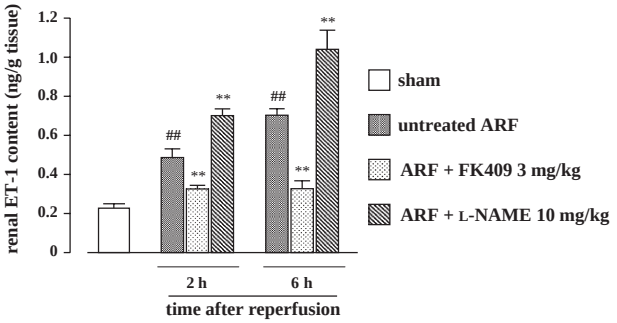

Fig. 6. Effect of FK409 or L-NAME on renal endothelin-1 contents at 2 and 6 h after reperfusion. FK409 or L-NAME was given intravenously 5 min before ischemia. Each column and bar represents the mean ± S.E.M. ($n = 6$). $^{\#\#}P < 0.01$, compared with sham-operated rats. $^{**}P < 0.01$, compared with untreated ARF rats. ARF, acute renal failure.

were measured in the absence or presence of FK409 injection (1, 3 mg/kg, i.v.). We obtained evidence that the administration of FK409 resulted in a dose-related increase in plasma NO_x concentration, which meant that FK409 released NO in the kidney during ischemia and immediately after reperfusion. We moreover asked if FK409 would improve the acute deterioration of renal function observed immediately after the reperfusion. However, pretreatment of FK409 failed to ameliorate the decreased responses to renal plasma flow and glomerular filtration rate, in contrast to the observation at 24 h after ischemia/reperfusion. These findings suggested that the FK409-induced improvement of impaired renal function and tissue damage, observed at 24 h after the ischemia/reperfusion, was not due to acute renal hemodynamic changes, which might occur with FK409-induced renal vasodilation. It seemed likely that NO released from FK409 improved postischemic acute renal failure by preventing abnormal events occurring during a 45-min ischemia and an early phase after reperfusion; however, the mechanisms by which FK409 exhibits protective effects on ischemic acute renal failure were not elucidated in the previous study. Mitsutomi et al. (1999) demonstrated that of the various NO donors used, NOR compounds (NOR2, NOR3, and NOR4) suppressed both endothelin-1 secretion and preproendothelin-1 mRNA expression in cultured vascular endothelial cells, and that NOR3, known as FK409, showed the most potent inhibitory effect on endothelin-1 production. Thus, utilization of FK409 is feasible for evaluation of roles of exogenous NO in the production of endothelin-1, one of the causal factors of the ischemia/reperfusion-induced acute renal failure. On the other hand, conventional NO synthase inhibitors such as L-NAME and L-NMMA have been used as pharmacological tools for examining the effect of endogenous NO on ischemic acute renal failure (Chintala et al., 1993; López-Neblina et al., 1995; Öztürk et al., 2001). The inhibition of endogenous NO production by L-NAME and L-NMMA was also found to increase endothelin-1 production at the transcriptional level in cultured endothelial cells (Mitsutomi et al., 1999). Based on these findings, we used FK409 and L-NAME to investigate whether the effects of exogenous and endogenous NO on ischemic acute renal failure would be related with ischemia/reperfusion-induced renal endothelin-1 overproduction.

The current study showed that FK409 was capable of preventing renal function impairment and tissue injuries, such as proteinaceous casts in tubuli, medullary congestion, and tubular necrosis, induced by ischemia/reperfusion. These results confirmed our previous findings that exogenous NO protects ischemia/reperfusion-induced renal injuries (Matsumura et al., 1998). The beneficial effects of FK409 on ischemic acute renal failure are in agreement with those obtained by using other NO donors such as sodium nitroprusside (López-Neblina et al., 1995; Sánchez-Pérez-Verdía et al., 2001) or molsidomine (Öztürk et al., 2001). In contrast, L-NAME aggravated renal injuries of acute renal failure rats. These deteriorative effects of L-NAME are similar to those obtained by Chintala et al. (1993) using L-NMMA. On the other hand, López-Neblina et al. (1995) and Öztürk et al. (2001) observed that NO synthase inhibition by L-NMMA and L-NAME did not affect ischemia/reperfusion-induced renal dysfunction and degeneration, respectively. The reason for this discrepancy is unknown, but differences in experimental protocol (e.g., surgical procedure, duration of ischemia, and given dose) may influence the efficacy of drugs. In the present study, more interesting observations were that FK409 reduced the ischemia/reperfusion-induced increases in renal endothelin-1 content, whereas L-NAME enhanced the increment of renal endothelin-1 content after reperfusion.

Shortly after the discovery of endothelin-1, this peptide has been considered to be an important mediator of acute renal failure because of its intense renal vasoconstrictive properties (Firth et al., 1988). It also has been shown that endothelin-1 mRNA expression and endothelin-1 content, and its affinity for ET receptors, are elevated in the postischemic kidney (Shibouta et al., 1990; Firth and Ratcliffe, 1992; Nambi et al., 1993; Wilhelm et al., 1999; Kuro et al., 2000). Moreover, pharmacological studies using endothelin-1 receptor antagonists (Mino et al., 1992; Gellai et al., 1995; Birck et al., 1998; Kuro et al., 2000) support the possibility of endothelin-1 as a causal factor of ischemic acute renal failure. A study by Wilhelm et al. (1999) indicated that initial endothelin-1 up-regulation in the kidney occurs secondary to the ischemia, but reperfusion contributes to sustaining this up-regulation. In addition, they observed a marked increase of endothelin-1 in the peritubular capillary network, suggesting that endothelin-1-induced vasoconstriction plays a pathophysiological role in ischemia/reperfusion-induced tubular necrosis. Taken together, it is likely that increased local production of endothelin-1 and its action occur in the kidney after reperfusion. In the present study, we also observed that renal endothelin-1 content in untreated acute renal failure rats increased significantly 2 h after reperfusion. This increase was more marked 6 h after reperfusion, and,

thereafter, the increased level appeared to decrease gradually but remained higher even at 24 h after reperfusion, compared with those in sham-operated control animals. The increases in endothelin-1 content at 2, 6, and 24 h after reperfusion were significantly reduced by pretreatment with FK409. These results suggest that the effectiveness of FK409 in the pathogenesis of ischemic acute renal failure is partly through the suppression of endothelin-1 overproduction in postischemic kidneys. On the other hand, L-NAME could not affect the increased endothelin-1 content at 24 h after reperfusion; however, the elevated renal endothelin-1 content at 2 and 6 h after reperfusion were augmented by pretreatment with the higher dose of L-NAME. From these observations, it is likely that pharmacological actions of L-NAME on the renal endothelin-1 production in ischemic acute renal failure rats would not be long-lasting. In addition, it is conceivable that an augmentation of L-NAME on the renal endothelin-1 overproduction enhanced in an early phase after reperfusion would potentiate endothelin-1 actions on the postischemic kidney, and exaggerate the renal function impairment and tissue injury that are observed 24 h after reperfusion.

It is difficult to determine what mechanisms are responsible for the inhibitory effect of NO on endothelin-1 production in an in vivo study of ischemic acute renal failure. We have recently obtained evidence that NO has a crucial role in the regulation of endothelin-1 production at transcriptional level, through the suppression of nuclear factor-κB (NF-κB) activation (Ohkita et al., 2002a,b), and that FK409 inhibits endothelin-1 production accompanying the suppression of NF-κB activation in cultured vascular endothelial cells (Ohkita et al., 2003). We also have demonstrated that NF-κB suppressors such as lactacystin (Itoh et al., 2001) and α-lipoic acid (Takaoka et al., 2002) prevent the development of ischemia/reperfusion-induced acute renal failure, and the effect is accompanied by suppression of the enhanced endothelin-1 production in the kidney. Taken together with the present study, one possible mechanism by which both exogenous and endogenous NO ameliorate renal dysfunction and degeneration in ischemic acute renal failure rats seems to be attributed to the inhibition of enhanced expression of renal endothelin-1 via NF-κB activation in an early phase after reperfusion.

NO is synthesized by different NO synthase isoforms, which have been cloned and characterized: endothelial NO synthase, neuronal NO synthase, and inducible NO synthase. It has been demonstrated that renal ischemia/reperfusion injury is efficiently attenuated by genetic deficiency or pharmacological blockade of inducible NO synthase (Ling et al., 1999; Walker et al., 2000; Chatterjee et al., 2002). While inducible NO synthase-derived NO predominantly elicits pathologic effects, endothelial NO synthase-derived NO has been believed to be responsible for maintaining physiologic renal hemodynamics and functions (Goligorsky and Noiri, 1999). In addition, we have recently observed that there is a marked impairment of renal function in endothelial NO synthase-deficient mice subjected to 45 min of ischemia, showing a tendency to further deterioration compared with wild-type mice (Yamasowa et al., 2005). Taken together with the present study indicating that L-NAME enhances the ischemia/reperfusion-induced renal endothelin-1 overproduction within 6 h of reperfusion and worsens renal injuries at 24 h after reperfusion, it seems likely that this nonselective NO synthase inhibitor strongly suppresses the production of NO generated by endothelial NO synthase rather than inducible NO synthase, in an early phase after reperfusion. Further experiments are required to clarify the role of NO derived from each NOS synthase in the endothelin-1 overproduction of postischemic kidneys.

In conclusion, our results indicate that NO exerts protective effects against ischemia/reperfusion-induced renal dysfunction and degeneration, and that suppressive effects of NO on the renal endothelin-1 overproduction induced by ischemia/reperfusion are probably responsible for the protective effect of NO against ischemic acute renal failure.

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