Protective Effect of FK409, a Spontaneous Nitric Oxide Releaser, on Ischemic Acute Renal Failure in Rats

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ABSTRACT

The contribution of nitric oxide (NO) to ischemic acute renal failure (ARF) is controversial. In the present study, we investigated the effect of FK409 ([±]-4-ethyl-2-[[E]-hydroxyiminio]-5-nitro-3-hexenamide), a spontaneous NO donor, on ischemic ARF in rats. Ischemic ARF was induced by occlusion of the left renal artery and vein for 45 min followed by reperfusion, 2 weeks after contralateral nephrectomy. Renal functional parameters such as blood urea nitrogen, plasma creatinine, creatinine clearance, urine flow, urinary osmolality and fractional excretion of sodium were measured to test the effectiveness of the drug. Renal function in untreated ARF rats markedly decreased at 24 hr after reperfusion and thereafter tended to recover gradually. Intravenous bolus injection of FK409 at a dose of 1 mg/kg before the occlusion markedly attenuated the ischemic ARF–induced decreases in renal function, to the same extent as verapamil (1 mg/kg i.v.). The protective effect of FK409, at a dose of 3 mg/kg, was much more potent than that of the lower dose. Histopathological examination of the kidney of untreated ARF rats revealed severe renal damages, such as tubular necrosis, proteinaceous casts in tubuli and medullary congestion. These renal damages were significantly attenuated by treatment with FK409, at each dose given and this attenuation exceeded that seen with verapamil treatment. FK 409 administration led to a dose-dependent increase in NO metabolites concentration in renal venous blood immediately after the reperfusion. These findings suggest that NO has a crucial role in the pathogenesis of ischemic ARF. Spontaneous NO donors may be clinically effective in cases of ischemic ARF.

In vascular endothelium, NO is synthesized from the amino acid l-arginine by the enzyme NO synthase (Moncada et al., 1991). This NO accounts for biological actions of endothelium-derived relaxing factor, and acts via stimulation of soluble guanylate cyclase in vascular smooth muscle cells (Moncada et al., 1991). The synthesis and release of NO at the basal level tonically contribute to the regulation of vascular tone in the cardiovasculard system, based on that inhibition of NO synthesis by N⁶-nitro-l-arginine and other arginine analogues induces a hypertensive response and decreases local blood flow in laboratory animals (Gardiner et al., 1990). In the kidney, intrarenal arterial infusion of NO synthase inhibitors causes potent renal vasoconstriction and antidiuresis (Egi et al., 1994). The NO synthase inhibitor impairs pressure-induced natriuresis and renal autoregulation, in anesthetized dogs (Salom et al., 1992; Majid et al., 1993), thereby suggesting that endogenous NO plays an important role in the regulation of renal vascular tone and renal tubular reabsorption of sodium and/or water.

In addition to the physiological importance of NO in the regulation of renal hemodynamics and tubular function, recent studies have demonstrated that changes in NO production and/or metabolism in the kidney are closely related to various renal pathological conditions, such as chronic renal failure with renal mass reduction, lipopolysaccharide-provoked renal dysfunction and ischemic ARF (Ashab et al., 1995; Schwartz et al., 1997; Caramelo et al., 1996).

The role of NO in ischemic ARF is controversial. Lieberthal et al. (1991) found that decreases in RPF and GFR in rats with hypovolemic shock induced by hemorrhage, were to some extent overcome by the inhibition of NO production. NO synthase inhibitor was reported to prevent hypoxia/reoxigenation injury in rat proximal tubules, thereby suggesting that NO is synthesized in proximal tubules and is involved in tubular hypoxia/reoxigenation injury (Yu et al., 1994). In contrast, Chintala et al. (1993) noted that the inhibition of NO production with a NO synthase inhibitor significantly deteriorated renal function of the postischemic kidney in anesthetized rats, whereas pretreatment with the NO precursor l-arginine abolished the NO synthase inhibitor-induced deterioration of renal function. Similar

Abbreviations: NO, nitric oxide; FK409, ([±]-4-ethyl-2-[[E]-hydroxyiminio]-5-nitro-3-hexenamide; ARF, acute renal failure; BUN, blood urea nitrogen; Pcr, plasma creatinine concentration; Ccr, creatinine clearance; UF, urine flow; Uosm, urinary osmolality; FE Na, fractional excretion of sodium; RPF, renal plasma flow; GFR, glomerular filtration rate; NOx, NO metabolites.
improvement by L-arginine against the decreased renal function in ischemic ARF was noted by Schramm et al. (1994), although they did not observe detrimental effects of the NO synthase inhibitor.

FK409, (±)-E-4-ethyl-2-[6-hydroxyiminio]-5-nitro-3-hexenamide, is a structurally unique vasodilator discovered from the fermentation product of Streptomyces grisseoroseus (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 isolated dog coronary arteries (Yamada et al., 1991) and the rat aorta (Isomoto et al., 1993). Furthermore, it has been reported that antiplatelet effects (Kita et al., 1994b) and antiinflammatory effects (Kita et al., 1994c) of FK409 are more potent than those of organic nitrates such as isosorbide dinitrate, these effects being based on the potential of spontaneous NO generation. Thus, utilization of this compound is feasible for evaluation of roles of NO in the pathogenesis of ischemic ARF. We examined the effect of FK409 on renal functional and the histological damages in ischemic ARF and effects of the drug were compared with those seen with the calcium channel blocker verapamil which ameliorates posts ischemic renal failure (Goldfarb et al., 1983).

Materials and Methods

Animals and experimental design

Experiment 1. Male Sprague-Dawley rats (280–320 g, 8 weeks of age, Japan SLC, Shizuka) were used. The animals were housed in a light controlled room with a 12-hr light/dark cycle and were allowed ad libitum access to food and water. Two weeks before the study (at 6 weeks of age), the right kidney was removed through a left ureter for urine collection. A 60- to 90-min period was allowed for stabilization of mean arterial pressure and UF. After the equilibration period, urine samples were collected during two 20 min control clearance periods. Results for the second control period served as basal values for renal function. Following the control periods, FK409 or its vehicle (0.9% saline) was administered intravenously by the slow bolus injection (volume, 1 ml/kg; duration, 2 min). Five min after the injection, left renal artery and vein were occluded with a nontraumatic clamp for 30 min. At the end of the ischemic period, the clamp was released for blood reperfusion. Urine samples were then collected during five consecutive 20 min periods (E1–E5). Blood samples (0.2 ml each) were obtained at 20 min before drug injection and at 45 min and 85 min after the injection, respectively. The blood loss was replaced by injecting an equal volume of blood from donor rats. Plasma was immediately separated by centrifugation. In preliminary experiments, no urine production was usually observed after 45 min of ischemia and reperfusion, therefore, the 30 min of ischemia was used.

Experiment 2. Some rats from each group separated as in Experiment 1 were killed 1 day after the 45-min ischemia and reperfusion, and their left kidneys were removed and processed for light microscopic observation, according to standard procedures. The kidneys were then preserved in phosphate-buffered 10% formalin, after which the kidneys were chopped into small pieces, embedded in paraffin wax, and cut at 3 μm and stained with hematoxylin and eosin. Histopathological changes were graded as no change (– or 0), mild (± or 1), moderate (+ or 2), severe (+ + or 3) and very severe (+ + + or 4) based on the microscopic observations of each section.

Experiment 3. In separate experiments, we examined the effect of FK409 (1 mg/kg i.v.) on the acute damages of renal function after ischemia and reperfusion. Animals were uninephrectomized, as described above. After a 2-week recovery period, the rats were anesthetized with sodium thiobutabarbital (Inactin, 100 mg/kg i.p.) and placed on a heated surgical tray that maintained rectal temperature between 37°C and 38°C. After tracheotomy, the right femoral artery and vein were cannulated to monitor arterial blood pressure and for infusion of 0.9% saline containing 1.0% inulin and 0.3% p-aminohippuric acid (0.02 ml/min), respectively. The right carotid artery and vein were also cannulated for blood sampling and for infusion of 2.5% mannitol/0.45% saline (0.08 ml/min), which ensures urine production after the ischemia, respectively. After making an abdominal midline incision, the left kidney was exposed. A polyethylene cannula was inserted into the left ureter for urine collection. A 60- to 90-min period was allowed for stabilization of mean arterial pressure and UF. After the equilibration period, urine samples were collected in two 20 min control clearance periods. Results for the second control period served as basal values for renal function. Following the control periods, FK409 or its vehicle (0.9% saline) was administered intravenously by the slow bolus injection (volume, 1 ml/kg; duration, 2 min). Five min after the injection, left renal artery and vein were occluded with a nontraumatic clamp for 30 min. At the end of the ischemic period, the clamp was released for blood reperfusion. Urine samples were then collected during five consecutive 20 min periods (E1–E5). Blood samples (0.2 ml each) were obtained at 20 min before drug injection and at 45 min and 85 min after the injection, respectively. The blood loss was replaced by injecting an equal volume of blood from donor rats. Plasma was immediately separated by centrifugation. In preliminary experiments, no urine production was usually observed after 45 min of ischemia and reperfusion, therefore, the 30 min of ischemia was used.

Blood and urine measurements

BUN and creatinine levels in plasma or urine were determined using the BUN-test-Wako and Creatinine-test-Wako (Wako Pure Chemical Industries, Osaka, Japan), respectively. Uosm was measured by freezing point depression (Fiske, MA). Urine and plasma sodium, PNa is the plasma sodium concentration. Urine and plasma sodium concentrations were determined using a flame photometer (Hitachi, 205D). FENa (%) was calculated from the formula; FENa = (UNaV/(PNa × UNaV)) × 100, where UNaV is urinary excretion of sodium, PNa is the plasma sodium concentration. Urine and plasma inulin levels were measured spectrophotometrically (Hitachi, 650–60) according to the method of Vurek and Pegram (1966). The GFR was estimated from the inulin clearance. Urine and plasma p-aminohippuric acid levels were measured by colorimetry according to the Bratton-Marshall method. The RPP was estimated from the p-aminohippuric acid clearance.
Drugs

FK409 (Fujisawa Pharmaceutical Co., Ltd.) and verapamil (Sigma Chemical Co., St. Louis, MO) were dissolved in saline (0.9%) just before administration. Other chemicals were obtained from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical Industries (Osaka, Japan).

Statistical analysis

Values were expressed as mean ± S.E.M. For statistical analysis, we used one-way ANOVA followed by a Bonferroni’s multiple comparison test (Renal functional studies in Experiment 1). Histological data in Experiment 2 were analysed using Kruskal-Wallis nonparametric test (Renal functional studies in Experiment 1). Histological examination revealed severe lesions in the kidney of untreated ARF rats (1 day after the 45-min ischemia). These changes were characterized by tubular necrosis, proteinaceous casts in tubuli, and medullary congestion and hemorrhage. Pretreatment with FK409 at 1 or 3 mg/kg prevented development of all these lesions. Verapamil tended to attenuate the histological damages, but its effect was less effective than those seen in FK409-treated animals (tables 3 and 4). Typical photographs of each group are shown in figures 3 through 5.

Results

Renal function after the ischemia and effects of FK409 and verapamil (Experiment 1). As shown in figures 1 and 2, renal function of rats subjected to 45-min ischemia showed a marked deterioration when measured 1 day after the reperfusion. As compared with sham-operated rats, untreated ARF rats showed significant increases in BUN (98.7 ± 9.6 vs. 25.2 ± 0.8 mg/dl), Pcr (2.71 ± 0.34 vs. 0.76 ± 0.06 mg/dl), UF (90.3 ± 11.2 vs. 38.2 ± 5.8 μl/min/kg) and FENa (2.80 ± 0.76 vs. 0.32 ± 0.04%) and significant decreases in Ccr (1.29 ± 0.30 vs. 496 ± 0.68 ml/min/kg) and Uosm (437 ± 47 vs. 1401 ± 88 mOsm/kg). The administration of FK409 in a dose of 1 mg/kg produced a significant preventive effect (except for changes in UF) against the ischemia-induced deterioration of renal function, but such effects were incomplete. When 3 mg/kg of FK409 was given, renal function changes induced by ischemia were abolished almost completely (BUN, 31.1 ± 1.7 mg/dl; Pcr, 0.84 ± 0.02 mg/dl; Ccr, 4.04 ± 0.19 ml/min/kg; UF, 32.8 ± 3.6 μl/min/kg; Uosm, 1355 ± 82 mOsm/kg; FENa, 0.30 ± 0.07%). As reported (Goldfarb et al., 1983), verapamil (1 mg/kg) also attenuated significantly the decreased responses of renal function to the ischemia, to a degree similar to those of the lower dose of FK409. The renal function of untreated ARF rats remained at an aggravated condition 2 days after the reperfusion (except for FENa) (table 1). Thereafter, renal function improved gradually and Ccr recovered to the level of the sham-operated control at 7 days (table 2). In contrast, sham operation had no detrimental effects on renal function over the 7-day observation period. Throughout the 7-day observation period, FK409 exerted a dose-related and marked attenuation of the functional impairment induced by the ischemia. The preventive effects of FK409 at the higher dose were potent, and values of renal function parameters were similar to those seen in sham-operated control. Verapamil also attenuated renal function changes at 2 and 7 days after reperfusion.

Histological renal damage after ischemia and effects of FK409 and verapamil (Experiment 2). Histopathological examination revealed severe lesions in the kidney of untreated ARF rats (1 day after the 45-min ischemia). These changes were characterized by tubular necrosis, proteinaceous casts in tubuli, and medullary congestion and hemorrhage. Pretreatment with FK409 at 1 or 3 mg/kg prevented development of all these lesions. Verapamil tended to attenuate the histological damages, but its effect was less effective than those seen in FK409-treated animals (tables 3 and 4). Typical photographs of each group are shown in figures 3 through 5.

Effects of FK409 on immediate renal function change after the ischemia and reperfusion (Experiment 3). Figure 6 shows immediate changes in renal function after 30-min ischemia followed by reperfusion, in vehicle- or FK409 (1 mg/kg i.v.)-treated anesthetized rats. In the vehicle-treated group, basal values of RPF, GFR and FENa averaged 3.23 ± 0.40 ml/min/g kidney wt., 0.85 ± 0.15 ml/min/g kidney wt. and 0.36 ± 0.15%, respectively. For the FK409-treated group, similar results were obtained (RPF, 2.97 ± 0.26 ml/min/g kidney wt.; GFR, 0.75 ± 0.07 ml/min/g kidney wt.; FENa, 0.33 ± 0.06%). During the first 20-min period after the reperfusion, the levels of RPF and GFR were

![Graphs showing effects of FK409 and verapamil on renal function](https://via.placeholder.com/150)

**Fig. 1.** Effects of FK409 and verapamil on blood urea nitrogen (BUN, A), plasma creatinine (Pcr, B) and creatinine clearance (Ccr, C) at 1 day after ischemia/reperfusion. Drugs were given intravenously 5 min before the ischemia (45 min). ARF, acute renal failure. Each column and bar represents the mean ± S.E.M. of seven rats. #P < .05, **P < .01, compared with untreated ARF; +P < .05, compared with sham rats.
Histopathological changes of kidneys in ARF rats

<table>
<thead>
<tr>
<th>Histopathological changes/grade</th>
<th>Sham (n = 5)</th>
<th>Untreated ARF (n = 5)</th>
<th>ARF + FK409 1 mg/kg (n = 6)</th>
<th>ARF + verapamil 1 mg/kg (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular necrosis</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Proteinaceous casts in tubuli</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medullary congestion</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All data are expressed as the number of animals with histopathological changes. Grades: no change (−), mild (+), moderate (++), severe (+++), very severe (+++). ARF, acute renal failure.

extremely low, in both groups (near zero). Thereafter, these renal hemodynamic parameters gradually increased over the 100-min observation period, in the same manner in both groups. The level of FENa in the vehicle-treated group was markedly elevated after the reperfusion, being 24.64 ± 5.60% in the second 20-min period. Thereafter, FENa gradually decreased over the observation period. FK409 significantly attenuated this excretory impairment, in contrast to changes

![Fig. 2. Effects of FK409 and verapamil on urine flow (UF, A), urinary osmolality (Uosm, B) and fractional excretion of sodium (FENa, C) at 1 day after ischemia/reperfusion. Drugs were given intravenously 5 min before the ischemia (45 min). ARF, acute renal failure. Each column and bar represents the mean ± S.E.M. of seven rats. *P < .05, **P < .01, compared with untreated ARF; #P < .01, compared with sham rats.]

### TABLE 1
Protective effect of FK409 at 2 days after ischemic acute renal failure

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>BUN (mg/dl)</th>
<th>Pcr (ml/min/kg)</th>
<th>Ccr (ml/min/kg)</th>
<th>UF (l/min/kg)</th>
<th>Uosm (mOsm/kg)</th>
<th>FENa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (n = 7)</td>
<td>24.7 ± 1.0</td>
<td>0.78 ± 0.01</td>
<td>4.00 ± 0.56</td>
<td>31.2 ± 5.3</td>
<td>1583 ± 80</td>
<td>0.51 ± 0.12</td>
</tr>
<tr>
<td>Untreated ARF (n = 7)</td>
<td>101.0 ± 14.4</td>
<td>2.35 ± 0.31</td>
<td>1.21 ± 0.13</td>
<td>71.0 ± 10.3</td>
<td>532 ± 72</td>
<td>0.80 ± 0.14</td>
</tr>
<tr>
<td>ARF + FK409 1 mg/kg (n = 7)</td>
<td>52.6 ± 9.8</td>
<td>1.24 ± 0.17</td>
<td>2.50 ± 0.29</td>
<td>48.5 ± 8.3</td>
<td>916 ± 137</td>
<td>0.43 ± 0.07</td>
</tr>
<tr>
<td>ARF + FK409 3 mg/kg (n = 7)</td>
<td>30.0 ± 1.7</td>
<td>0.86 ± 0.05</td>
<td>3.87 ± 0.60</td>
<td>32.8 ± 3.8</td>
<td>1368 ± 96</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>ARF + verapamil 1 mg/kg (n = 7)</td>
<td>46.0 ± 3.8</td>
<td>1.12 ± 0.06</td>
<td>2.98 ± 0.19</td>
<td>53.1 ± 6.8</td>
<td>897 ± 119</td>
<td>0.35 ± 0.05</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. Drugs were intravenously injected 5 min before the ischemia (45 min). At 2 days after the reperfusion, 5-hr urine was collected. *P < .05, **P < .01, compared with untreated ARF; #P < .01, compared with sham rats. ARF, acute renal failure.

### TABLE 2
Protective effect of FK409 at 7 days after ischemic acute renal failure

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>BUN (mg/dl)</th>
<th>Pcr (ml/min/kg)</th>
<th>Ccr (ml/min/kg)</th>
<th>UF (l/min/kg)</th>
<th>Uosm (mOsm/kg)</th>
<th>FENa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (n = 7)</td>
<td>26.4 ± 1.6</td>
<td>0.83 ± 0.01</td>
<td>3.89 ± 0.27</td>
<td>34.9 ± 5.6</td>
<td>1283 ± 96</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>Untreated ARF (n = 7)</td>
<td>42.2 ± 5.7</td>
<td>1.06 ± 0.07</td>
<td>3.99 ± 0.54</td>
<td>74.5 ± 9.7</td>
<td>708 ± 51</td>
<td>0.38 ± 0.10</td>
</tr>
<tr>
<td>ARF + FK409 1 mg/kg (n = 7)</td>
<td>30.4 ± 1.8</td>
<td>0.93 ± 0.08</td>
<td>4.22 ± 0.98</td>
<td>49.0 ± 7.1</td>
<td>1106 ± 95</td>
<td>0.46 ± 0.10</td>
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<tr>
<td>ARF + FK409 3 mg/kg (n = 7)</td>
<td>28.3 ± 2.2</td>
<td>0.85 ± 0.04</td>
<td>3.92 ± 0.32</td>
<td>33.5 ± 3.2</td>
<td>1368 ± 91</td>
<td>0.16 ± 0.06</td>
</tr>
<tr>
<td>ARF + verapamil 1 mg/kg (n = 7)</td>
<td>26.0 ± 1.6</td>
<td>0.87 ± 0.05</td>
<td>4.31 ± 0.38</td>
<td>50.4 ± 7.8</td>
<td>1095 ± 127</td>
<td>0.35 ± 0.04</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. Drugs were intravenously injected 5 min before the ischemia (45 min). At 7 days after the reperfusion, 5-hr urine was collected. *P < .05, **P < .01, compared with untreated ARF; #P < .05, #P < .01, compared with sham rats. ARF, acute renal failure.

### TABLE 3
Histopathological changes of kidneys in ARF rats

All data are expressed as the number of animals with histopathological changes. Grades: no change (−), mild (+), moderate (++), severe (+++), very severe (+++). ARF, acute renal failure.
Values of FENa in the first 20-min period could not be calculated because of no urine output.

Plasma NOx levels in renal vein (Experiment 4).

Blood samples were taken from the renal vein immediately after reperfusion, with or without FK409 pretreatment, and NOx concentrations were determined. As shown in Table 5, the administration of FK409 resulted in a dose-related increase in plasma NOx concentration, which meant that FK409 released NO during ischemia in the kidney.

Discussion

In the present study, we obtained evidence that FK409, a spontaneous NO releaser, markedly overcame the ischemia/reperfusion-induced impairment of renal function in rats. Histological renal damage induced by this posts ischemic ARF were also prevented by treatment with FK409, and to a lesser extent with verapamil, a calcium antagonist which has been reported to attenuate the posts ischemic ARF (Goldfarb et al., 1983).

Recent studies indicated that FK409-induced biological actions such as vasorelaxation and antiplatelet effect are mediated by NO, liberated by the decomposition of FK409 (Kita et al., 1994a, b). In the present study, we noted that the administration of FK409 before the ischemia resulted in dose-related increases in plasma NOx concentration in renal venous plasma obtained immediately after the reperfusion. Thus, it is reasonable to consider that NO formation in the kidney during the ischemia contributes to the drug-induced improvement of the posts ischemic ARF.

It has been reported that the oral dosing of FK409 (3.2 mg/kg) produces a significant hypotension only during the 20 min after the administration (Kita et al., 1997). We also found that the decreasing effect of FK409 (3 mg/kg, bolus i.v.) on blood pressure disappeared about 30 min after the injection (data not shown). We recently reported that intrarenal arterial infusion of FK409 to anesthetized rats led to renal vasodilation and diuresis (Uraye et al., 1997). Therefore, we asked if FK409 would improve the acute deterioration of renal function observed immediately after the reperfusion. However, pretreatment with FK409 failed to ameliorate the decreased responses of RPF and GFR, in contrast to the observation at 1 day after the ischemia/reperfusion.

Each value represents the mean ± S.E.M.

Table 4: Effects of FK409 and verapamil on histopathological changes of kidneys in ARF rats

<table>
<thead>
<tr>
<th>Tubular necrosis</th>
<th>Proteinaceous casts</th>
<th>Medullary congestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated ARF (n = 5)</td>
<td>3.40 ± 0.24</td>
<td>2.80 ± 0.20</td>
</tr>
<tr>
<td>ARF + FK409 1 mg/kg (n = 6)</td>
<td>1.50 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.17 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARF + FK409 3 mg/kg (n = 6)</td>
<td>1.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.50 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARF + verapamil 1 mg/kg (n = 6)</td>
<td>2.67 ± 0.33</td>
<td>2.33 ± 0.33</td>
</tr>
</tbody>
</table>

Grades: no change (0), mild (1), moderate (2), severe (3), very severe (4).

<sup>a</sup> P < .05, <sup>b</sup> P < .01, compared with untreated ARF.

ARF, acute renal failure.

Fig. 3. Light microscopy of outer zone outer stripe of medulla of the kidney treated with vehicle (b), verapamil 1 mg/kg (c), FK409 1 mg/kg (d) or FK409 3 mg/kg (e), 1 day after the ischemia/reperfusion, and sham rat (a). Drugs were given intravenously 5 min before the ischemia (45 min). Arrows indicate tubular necrosis (hematoxylin and eosin staining, magnification ×200).
findings suggest that the FK409-induced improvement of impaired renal function and tissue damages, observed at 1, 2 and 7 days after the ischemia/reperfusion, is not due to acute renal hemodynamic changes, which may be occur with the drug-induced renal vasodilation. On the other hand, marked elevation of FENa immediately after the ischemia/reperfusion was significantly attenuated by FK409 Thus, exogenous NO can improve also the acute tubular dysfunction induced...
by the ischemia/reperfusion. We suggest that the decreased formation and/or increased degradation of endogenous NO may occur during the ischemia/reperfusion. As NO is an extremely short-lived substance (Moncada et al., 1991), it seems likely that FK409 improves postischemic ARF by preventing abnormal events occurring during ischemia and/or immediately after the reperfusion.

Numerous attempts have been made to prevent postischemic ARF. Calcium antagonists (Goldfarb et al., 1983; Shimizu et al., 1990), endothelin receptor antagonists (Gellai et al., 1994; Chan et al., 1994) and other vasoactive substances such as atrial natriuretic peptides (Pollock and Opgenorth, 1990) were reported to attenuate the ischemia-induced impairment of renal function. However, the pathophysiological mechanism underlying the development and maintenance of the postischemic ARF remains obscure. Recent studies indicated that decreased endothelium-dependent vasorelaxation and NO production are related to an impaired renal function observed after ischemia/reperfusion (Conger et al., 1988; Cristol et al., 1993). NO precursor L-arginine has been reported to ameliorate postischemic ARF (Schramm et al., 1994). Furthermore, inhibition of NO synthase was seen to aggravate the postischemic ARF (Chintala et al., 1993). 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 peroxynitrates (Pryor and Squadrito, 1995).

Thus, although the role of NO in the pathogenesis of postischemic ARF is controversial, a recent investigation has found that a NO donor, sodium nitroprusside, prevents the neutrophil-mediated ischemic ARF, determined using isolated perfused rat kidneys (Linas et al., 1996). Neutrophils appear 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. On the other hand, it was reported that monoclonal antibodies to neutrophil adhesion molecules decrease the renal injury in postischemic ARF (Rabb et al., 1994). NO has been reported to inhibit neutrophil-related cellular events. Clancy et al. (1992) found that NO decreased superoxide anion production in neutrophils by inhibiting NADPH oxidase activity. Kubo et al. (1991) found that neutrophil adhesion in postcapillary venules was markedly enhanced by a NO synthase inhibitor and that the inhibitor-induced enhancement was prevented by L-arginine, thereby suggesting that NO may be an important endogenous inhibitor of neutrophil adhesion in venules. Taken together, an inhibitory effect on neutrophil-related cellular events may account for the FK409-induced improvement of the postischemic ARF.

Recent studies indicated that endothelin may be an important deleterious mediator in the pathogenesis of the postischemic ARF, based on findings that endothelin mRNA expression is markedly enhanced in the postischemic kidney (Firth and Ratcliffe, 1992), and that an endothelin-receptor antagonist (Gellai et al., 1994; Chan et al., 1994) or the endothelin-converting enzyme inhibitor phosphoramidon (Vemulapalli et al., 1993) prevents postischemic renal damages such as the decreases in RPF and GFR, and tubular dysfunction. It was stated that an inhibitor of NO synthase exerts an increased release of endothelin from cultured endothelial cells, thereby suggesting a role for endogenous NO as an inhibitory modulator on endothelin production (Boulanger and Lüscher, 1990). We found that FK409 suppressed the production of endothelin in endothelial cells (Takada et al., 1996). Thus, attenuation of endothelin production in the ischemic kidney may be partly involved in the FK409-induced improvement of the postischemic ARF.

Detrimental and beneficial effects of endogenous NO inhibition on ischemia/reperfusion-induced renal injury have

### TABLE 5

Plasma NOx concentration in renal vein after reperfusion following 45-min occlusion in anesthetized rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NOx Base line</th>
<th>After reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/ml</td>
<td></td>
</tr>
<tr>
<td>Vehicle (n = 3)</td>
<td>8.9</td>
<td>9.7</td>
</tr>
<tr>
<td>FK409 1 mg/kg (n = 3)</td>
<td>11.1</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>14.9</td>
<td>25.3</td>
</tr>
<tr>
<td></td>
<td>13.4</td>
<td>23.8</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>19.3</td>
</tr>
<tr>
<td>FK409 3 mg/kg (n = 3)</td>
<td>11.9</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>19.3</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Each value represents data on an individual rat. NOx, nitrites/nitrates.
been described (Chintala et al., 1993; Yu et al., 1994), and an exogenous NO precursor or NO donor produces bidirectional effects on this injury (Schramm et al., 1994; Yu et al., 1994; López-Neblina et al., 1995). Thus, although further attempts are required to clarify the role of NO in the pathogenesis of the ischemia/reperfusion injury, our findings clearly indicate the beneficial effect of a spontaneous NO releaser FK409 on the impairment of renal function and tissue damages observed in postischemic ARF in rats. We also suggest that the FK409-induced improvement of renal damages is closely related to the tubular epithelial function rather than the damage-induced renal hemodynamic change. Whether or not spontaneous NO donors such as FK409 are useful as protective agents against ischemic ARF in humans warrants attention.

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References


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