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Phosphodiesterase type 5 inhibition ameliorates nephrotoxicity induced by cyclosporin A in spontaneous hypertensive rats

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Abstract

Our recent study suggests that there is a reciprocal mechanism to maintain cGMP content, via both a decrease in cGMP degradation (decrease in cGMP-phosphodiesterase activity) and an increase in synthesis of cGMP (increase in guanylate cyclase activity) in the kidney of cyclosporin A-treated rats. We undertook this study to clarify the role of cGMP-phosphodiesterase in cyclosporin A nephrotoxicity by evaluating *N*-(3,4-dimethoxybenzyl)-2-{[(1*R*)-2-hydroxy-1-methylethyl]amino}-5-nitrobenzamide (FR226807), a phosphodiesterase type 5 inhibitor, in an animal model. Male spontaneous hypertensive rats (SHR) were treated with cyclosporin A (50 mg/kg) for 2 weeks or with cyclosporin A and FR226807 (3.2 mg/kg or 10 mg/kg) for 2 weeks. Cyclosporin A-treated rats showed renal dysfunction and histological change compared with vehicle-treated rats. Administration of FR226807 improved the renal dysfunction (increase in serum creatinine and fractional excretion of sodium, and decrease in creatinine clearance) as well as the pathological changes (tubular vacuolization) induced by cyclosporin A in SHR. At the molecular level, administration of FR226807 resulted in a further increase in cGMP content in the kidney, aorta and platelets from cyclosporin A-treated rats. Our present study demonstrates that cGMP-phosphodiesterase plays an important role in the cyclosporin A nephrotoxicity and also suggests that further inhibition of cGMP-phosphodiesterase is a potential pharmacological target for preventing cyclosporin A nephrotoxicity.

Keywords: cGMP; Phosphodiesterase type 5 inhibitor; FR226807

1. Introduction

Cyclosporin A is a potent and effective immunosuppressive agent, widely used to prevent rejection of transplanted organs and to treat autoimmune diseases, such as psoriasis and rheumatoid arthritis. However, its use is often limited by adverse effects, including nephrotoxicity (Myers, 1986). Cyclosporin A nephrotoxicity has been characterized by reduction in glomerular filtration rate, resulting in elevated serum creatinine levels and a decrease in creatinine clearance, and reduction in Na^+ and K^+ excretion.

The exact mechanism of cyclosporin A nephrotoxicity remains unclear, however, several studies have suggested that attenuation of the nitric oxide (NO) pathway may be involved in renal dysfunction induced by cyclosporin A. NO plays an important role in many physiological processes, and manipulation of the NO pathway may produce major medical benefits. In the kidney, both vascular and tubular effects of NO have been observed and NO contributes to hemodynamic regulation (Kurtz and Wagner, 1998; Schnermann, 1998; Persson and Bachmann, 2000). NO released from the endothelium to smooth muscle cells activates soluble guanylate cyclase, which catalyzes the conversion of GTP to the signaling molecule cGMP (Lucas et al., 2000). Intracellular cGMP concentration is regulated by not only guanylate cyclase but also cGMP-phosphodiesterase, which hydrolyzes cGMP to 5′-GMP (Beavo, 1995; Soderling and Beavo, 2000).

Recently, we demonstrated an increase in cGMP content, despite a decrease in NO synthase (NOS) activity and excretion of NO*x* in kidney, with fully expressed cyclosporin A nephrotoxicity. This increase in cGMP was brought on by both an increase in soluble guanylate cyclase and particle guanylate cyclase activity, and a decrease in

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activity of cGMP-phosphodiesterases, although the increase appears inadequate to prevent progression of cyclosporin A nephrotoxicity (Hosogai et al., 2001a). This suggests that further increases in cGMP content by an appropriate pharmacological agent might produce favorable effects for cyclosporin A nephrotoxicity.

The superfamily of mammalian phosphodiesterase isoenzymes consists of 11 families classified according to chromatographic properties, substrate specificity and susceptibility to inactivation by inhibitors (Beavo, 1995; Fisher et al., 1998a,b; Fawcett et al., 2000). In kidney, cGMP is mainly hydrolyzed by two isozymes, phosphodiesterase type 5, which is more abundant than other phosphodiesterase such as phosphodiesterase type 1 (Dousa, 1999). The present study was undertaken to ascertain whether a selective phosphodiesterase type 5 inhibitor, *N*-(3,4-dimethoxybenzyl)-2-{[(1*R*)-2-hydroxy-1-methylethyl]amino}-5-nitrobenzamide (FR226807) (Hosogai et al., 2001b), produces further increases in cGMP content and ameliorates cyclosporin A induced renal dysfunction.

2. Methods

All animal experiments were conducted in accordance with the recommendations for the Declaration of Helsinki, and internationally accepted principles in the care and use of experimental animals.

2.1. Experimental protocols

Male spontaneous hypertensive rats (SHR) weighing 300–350 g were purchased from Charles River, Japan. Animals were housed in a climate-controlled, light-regulated space with a 12-h light/dark cycle for at least 1 week before the experiment. They were allowed free access to rat chow and water. Body weight-matched animals were divided into four groups: (1) Control group (vehicle), (2) cyclosporin A group (50 mg/kg), (3) cyclosporin A group receiving 3.2 mg/kg FR226807 and (4) cyclosporin A group receiving 10 mg/kg FR226807. Cyclosporin A was administered orally once a day and FR226807 orally twice a day, both for 14 days. Unless stated otherwise, all experimental groups comprised eight animals.

2.2. Collection of samples

On the 14th day of drug administration, 24-h urine samples were collected in metabolic cages. The urine samples were stored at −80 °C until biochemical analysis was performed. The following day, rats were anesthetized with ether. Blood samples were collected into a tube containing heparin by subclavian vein puncture. They were then immediately centrifuged, and plasma was separated and frozen at −80 °C until processed. The left kidney was fixed in phosphate-buffered 10% formalin, and the right kidney and aorta were excised and frozen in liquid nitrogen, then kept at −80 °C until measurement of parameters. Kidney and aorta were homogenized in 1 ml of ice cold 20 mM HEPES buffer containing 0.32 M sucrose, 0.5 mM EDTA, 1 mM dithiothreitol and protease inhibitors (3 μM leupeptin, 1 μM pepstatin A and 1 mM phenylmethyl sulfonylfluoride) at pH 7.2 using a Polytron® homogenizer. The cytosol and particulate fractions were separated by ultracentrifugation at 12,500 × *g* for 60 min, at 4 °C.

2.3. Biochemical determinations

Plasma and urinary creatinine, Na^+ and K^+ levels were measured with an autoanalyzer (80FR, Hitachi). Creatinine clearance and fractional excretion of Na^+ were calculated using standard laboratory methods.

2.4. Histological studies

The left kidney was fixed in phosphate-buffered 10% formalin, processed and embedded in paraffin, then cut into 5-μm sections using conventional techniques. Sections were stained with periodic acid-Schiff's reagent and hematoxylin-eosin. Whole colored slides, containing at least 100 glomeruli each, were analyzed. Morphological analyses were blindly performed and analyzed using a semiquantitative scale with values from 0 to 4 for each of the following alterations: for tubular vacuolization, 1 = scant, 2 = slight, 3 = moderate, 4 = abundant and diffuse.

2.5. Measurement of cGMP content

Cytosol fractions were acetylated and cGMP levels were measured using a cyclic GMP [^{125}I] assay system® (Amersham International, IL, USA). The quantitative determination limit of this assay was 0.5 fmol. cGMP concentration was expressed per mg of protein.

2.6. NOS activity

NOS activity was measured using the method of Lugg et al. (1995), as NO formation determined by the conversion of [^{3}H]arginine to [^{3}H]citrulline. The cytosol fraction was passed through Dowex AG50WX-8 (Na^+) resin to remove endogenous arginine, and 50 μl aliquots were incubated for 60 min at 37 °C in the presence of 2 μCi/ml L-[^{3}H]arginine, 2 mM NADPH, 0.45 mM $CaCl_2$, 100 μM L-arginine and 10 μg/ml calmodulin, with or without 2 mM *N*-nitro-L-arginine methyl ester (L-NAME) or 5 mM EGTA. Non-specific NOS activity was determined by L-NAME, an inhibitor of NOS, and Ca^{2+} independency, a feature of inducible NOS (iNOS), was ascertained using EGTA, a Ca^{2+} chelator. After elimination of residual L-[^{3}H]arginine through the resin, L-[^{3}H]citrulline produced was measured using a scintillation counter. Protein concentrations in the cytosol were assayed with Bio-Rad protein assay reagent®. All values were

expressed per mg of protein. The Ca^{2+}-dependent NOS (constitutive NOS) activity was calculated from the difference between the amount of L-[^{3}H]citrulline formed in control tubes and the amount formed in tubes incubated with EGTA. The iNOS activity was calculated from the difference between the amount of L-[^{3}H]citrulline formed in tubes incubated with EGTA and the amount formed in tubes incubated with EGTA plus L-NAME.

2.7. Measurement of cGMP-phosphodiesterase activity

cGMP-phosphodiesterase activity of the cytosol fractions from kidney was determined using a modification of the two step radioisotope procedure (Thompson and Appleman, 1971). The reaction mixture (250 μl total volume) contained the cytosol fraction, 0.1 μM [^{3}H]cGMP, 30 mM $MgCl_2$, 1 mM dithiothreitol in 50 mM Tris–HCl (pH 8.0) buffer, with or without 50 mM isobutylmethylxanthine (IBMX), 50 mM EDTA or 40 mM $CaCl_2$ plus 10 unit/ml calmodulin. The reaction was initiated by addition of radiolabeled substrate and incubated in a water bath at 30 °C for 10 min, then stopped by boiling at 100 °C for 1 min. Reaction mixtures were incubated with 2 mg/ml snake venom to hydrolyze GMP to guanine for 10 min at 30 °C. After the addition of anion exchanger, the reaction mixture was vortex-mixed and centrifuged at 300 × *g* for 10 min. The resulting supernatant was transferred to Lumaplate®, and the [^{3}H]5-mononucleotide formed by hydrolysis of cyclic nucleotide was determined by Top counter®. The cGMP-specific cGMP-phosphodiesterase activity was calculated as the difference between the amount of [^{3}H]5-mononucleotide formed in tubes incubated with EDTA alone and the amount formed in tubes incubated with EDTA plus IBMX.

2.8. Measurement of guanylate cyclase activity

The cytosol fraction from kidney was fractionated further by centrifuging at 100 000 × *g* for 60 min at 4 °C. The resulting supernatant was collected and used as the cytosolic fraction. Soluble guanylate cyclase activity was measured by the method of Kimura et al. (1975) with slight modifications. Reaction mixture containing 50 mM Tris–HCl (pH 7.6), 0.5 mM IBMX, 3.5 mM creatine phosphate, 2.5 units/tube creatine phosphokinase and 50 μl of the cytosolic fraction were preincubated for 10 min at 37 °C. The assay was initiated by the addition of 4 mM $MgCl_2$ and 1 mM GTP in a final volume of 100 μl, and incubated for 15 min at 37 °C. The reaction was terminated by addition of 0.9 ml of 50 mM sodium acetate buffer (pH 4.0) to prevent nonenzymatic formation of cGMP, and heated at 90 °C for 3 min. cGMP was determined using a radioimmunoassay kit (cyclic GMP[^{125}I] assay system®, Amersham International). Particulate guanylate cyclase activity was measured by the same procedure as for soluble guanylate cyclase, except that 1 mM ATP was added to the working solution. The activity was corrected for protein content.

2.9. Drugs

Cyclosporin A and FR226807 were synthesized by Fujisawa Pharmaceutical, Japan. L-NAME, EGTA, EDTA, dithiothreitol, leupeptin, pepstatin A, phenylmethyl sulfonylfluoride, L-arginine, NADPH, calmodulin, GTP, snake venom, isobutylmethylxanthine, creatine phosphate, creatine phosphokinase, FAD, nitrate reductase and sodium acetate were purchased from Sigma. [^{3}H]cGMP and L-[^{3}H]arginine were purchased from NEN Life Science Products. All other chemicals were of the purest commercially available grade.

2.10. Statistical analysis

Data are presented as mean ± S.E. The difference between the control group and cyclosporin A group was analyzed using unpaired Student's *t*-test. The statistical significance of differences between the cyclosporin A treatment groups was determined by analysis of variance followed by Dunnett's multicomparison test. The score from light microscopy was compared using the Kruskall–Wallis test.

3. Results

3.1. Body weight, urine volume and kidney weight

Cyclosporin A decreased body weight during the 2-week treatment period ($P < 0.01$ vs. control; Table 1). Cyclosporin A-treated rats lost 54.2 g, whereas the control group gained 21.9 g. FR226807 attenuated the cyclosporin A-induced loss of body weight. There were no differences in urine volume and the ratio of kidney wet weight to body weight between treatment groups (Table 1).

Table 1
Body weight, urine volume and kidney weight in experimental groups

	Vehicle group	Cyclosporin A group	Cyclosporin A+ FR226807 3.2 mg/kg	Cyclosporin A+ FR226807 10 mg/kg
Body weight (g)				
Initial	321.3±9.9	320.2±4.0	318.0±6.4	324.8±4.1
Final	343.2±7.7	266.0±6.1[a]	275.4±6.2	299.7±3.5[b]
Urine volume (ml)	23.8±6.6	19.3±2.8	22.0±4.5	25.2±5.0
Kidney wet weight (g/kg)	6.5±0.4	6.8±0.1	6.7±0.3	6.8±0.2

Values are expressed as mean±S.E. CsA, cyclosporin A.
[a] $p<0.001$ vs. control group.
[b] $p<0.01$ vs. Cyclosporin A group.

3.2. Renal function

Fig. 1 shows renal function in the experimental groups. Two weeks of treatment with cyclosporin A significantly reduced creatinine clearance levels (0.6 ± 0.5 vs. 0.1 ± 0.2 ml/min/100 g, control vs. cyclosporin A group), and significantly increased both serum creatinine levels (0.34 ± 0.04 vs. 0.78 ± 0.05 mg/dl, control vs. cyclosporin A group) and fractional excretion of sodium (0.25 ± 0.05 vs. 0.72 ± 0.09%, control vs. cyclosporin A group) compared to the control group ($P < 0.001$). Simultaneous administration of FR226807 with cyclosporin A significantly prevented the decrease in renal function induced by cyclosporin A.

3.3. Histological studies

Fig. 2 shows representative light microscopic images of renal cortex from this study. Fig. 2A shows a renal cortex from control rat. Cyclosporin A caused hypertrophy of the afferent arteriole and Bowman's capsule, abundant and focal proximal tubular vacuolization and distension of the distal

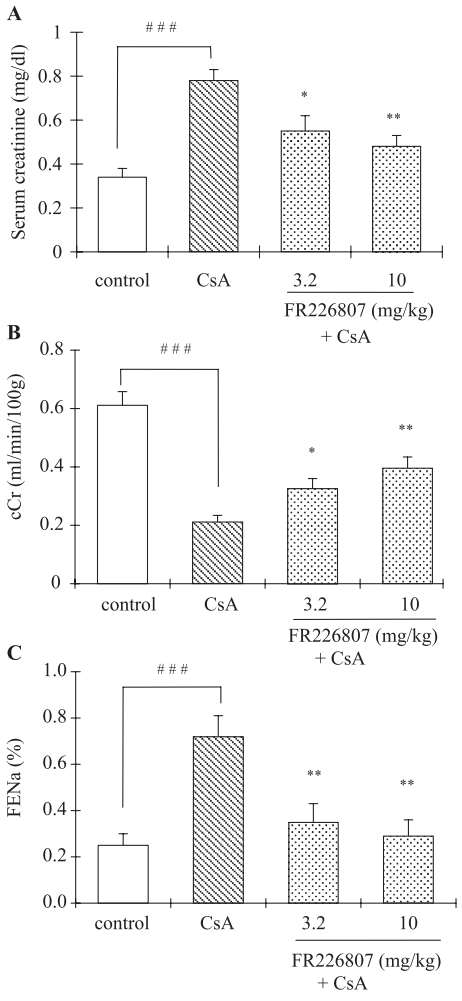

Fig. 1. Effect of FR226807 on cyclosporin A-induced change in serum creatinine (A), creatinine clearance (B), and fractional excretion of sodium (C). CsA: cyclosporin A. Results are expressed as mean ± S.E; $N = 7–8$. *$P < 0.05$, **$P < 0.01$ vs. cyclosporin A group. ###$P < 0.001$ vs. control group.

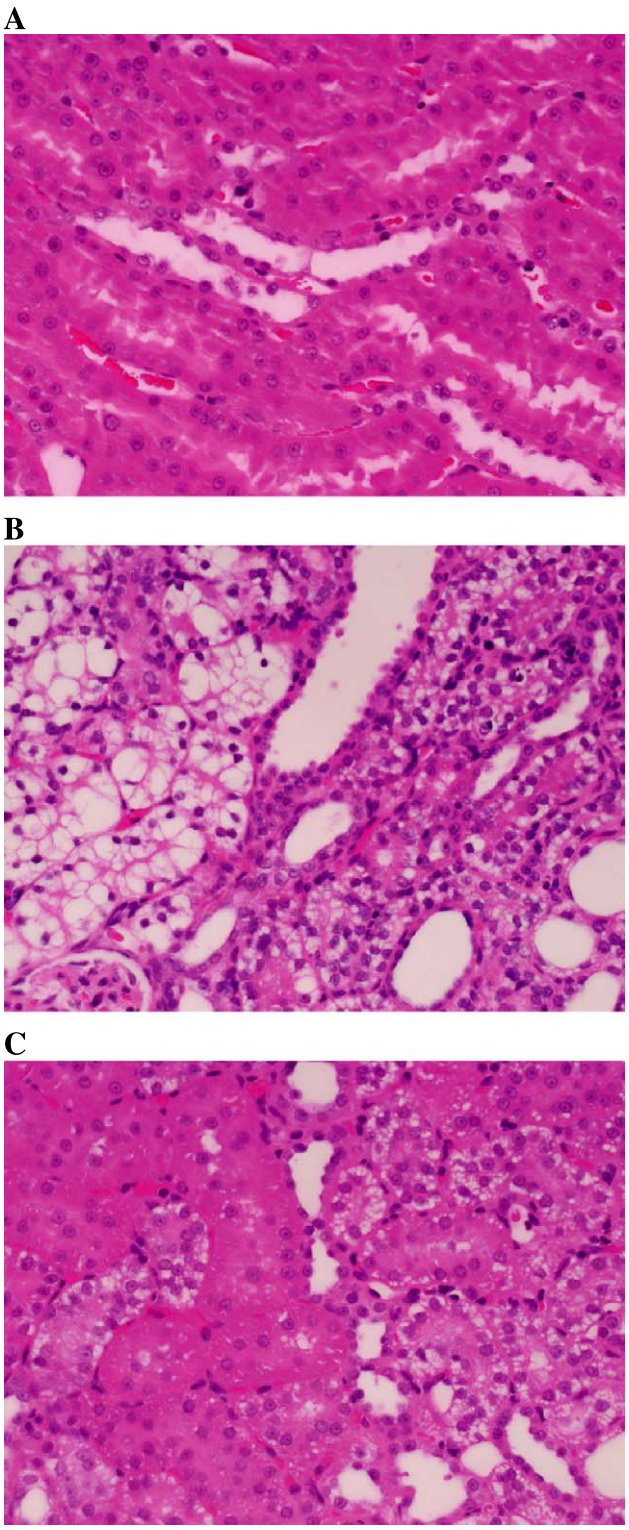

Fig. 2. Typical images of hematoxylin eosin-stained renal cortex from control (A), from cyclosporin A group (B) and from FR226807 group (C). Original magnification × 400.

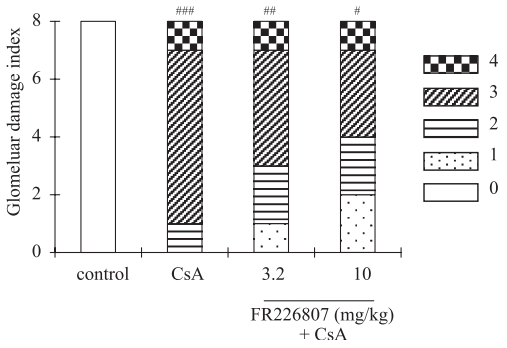

Fig. 3. Semiquantitative scoring by light microscopy in the experimental groups. CsA: cyclosporin A. $^{###}P<0.001$, $^{##}P<0.01$, $^{#}P<0.05$ vs. control group.

tubule (Fig. 2B). These pathological changes were largely blocked by treatment with FR226807 (Fig. 2C). Fig. 3 demonstrates the semiquantitative scoring of the tubular vacuolization index. Mean score of tubular vacuolization for the cyclosporin A group was 3, whereas for the control group, it was 0. FR226807 dose-dependently decreased the number of animals with a score of 3 and increased the number of animals with scores of 1 and 2.

3.4. cGMP content in kidney, aorta and platelets

As shown in Table 2, cGMP content in the kidney and aorta isolated from cyclosporin A-treated rat was 1.6-fold and 1.2-fold higher, respectively, than that for control rat. There was no difference in the cGMP content in platelets between the cyclosporin A and control groups. FR226807, at a dose of 10 mg/kg, significantly increased cGMP content in kidney and tended to dose-dependently increase the cGMP content in aorta and platelets.

3.5. cGMP-phosphodiesterase activity

Two weeks of treatment with cyclosporin A significantly reduced cGMP-specific cGMP-phosphodiesterase activity (29.6 ± 10.9 vs. 1.7 ± 0.5 fmol hydrolyzed cGMP/min/mg protein, control vs. cyclosporin A group). FR226807 dose-dependently decreased cGMP-phosphodiesterase activity and no cGMP-phosphodiesterase activity was detected in 10 mg/kg FR226807-treated groups.

Table 2
Effect of FR226807 on cGMP content (fmol/mg protein) in kidney, aorta and platelets

	Vehicle group	Cyclosporin A group	Cyclosporin A+ FR226807 3.2 mg/kg	Cyclosporin A+ FR226807 10 mg/kg
Kidney	3.82±0.28	5.98±0.27[a]	6.76±0.35[a]	7.18±0.35[a,b]
Aorta	111.2±13.5	135.0±20.7	147.4±15.2	154.4±22.9
Platelets	112.5±22.2	104.0±18.3	113.7±34.2	130.8±18.2

Values are expressed as mean±S.E. CsA, cyclosporin A.
[a] $p<0.01$ vs. control group.
[b] $p<0.05$ vs. Cyclsoporin A group.

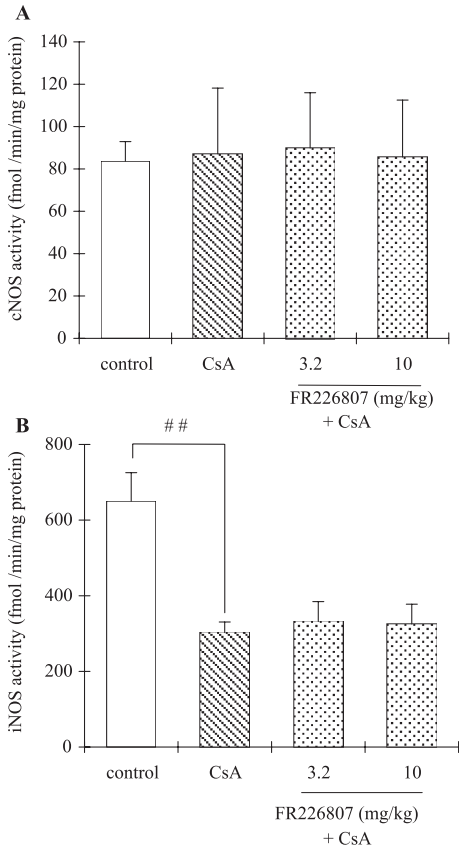

Fig. 4. Effect of FR226807 on cNOS activity (A) and iNOS activity (B) in kidney. CsA: cyclosporin A. Results are expressed as mean ± S.E; $N=7-8$. $^{##}P<0.01$ vs. control group.

3.6. NOS activity and guanylate cyclase activity

Cyclosporin A significantly decreased iNOS activity but not cNOS activity (Fig. 4). FR226807 had no effect on either iNOS or cNOS activities. Cyclosporin A significantly increased guanylate cyclase activity (Fig. 5). FR226807 had no effect on guanylate cyclase activity.

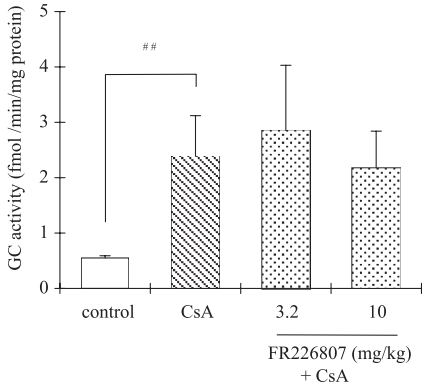

Fig. 5. Effect of FR226807 on guanylate cyclase activity in kidney. CsA: cyclosporin A. Results are expressed as mean ± S.E; $N=7-8$. $^{##}P<0.01$ vs. control group.

4. Discussion

We demonstrated that FR226807, a cGMP-phosphodiesterase type 5 inhibitor, improved cyclosporin A-induced nephrotoxicity in SHR. This effect may be associated with an increase in cGMP in kidney. The present study suggests the therapeutic utility of a phosphodiesterase type 5 inhibitor in cyclosporin A nephrotoxicity.

Cyclosporin A nephrotoxicity is characterized by two sites of injury: the glomerulus and the proximal tubule (Ader and Rostaing, 1998). Recently, we reported a model of cyclosporin A nephrotoxicity in SHR (Hosogai et al., 2001a) that has similarities to cyclosporin A nephrotoxicity in humans. Experiments were done on SHR, since normotensive rats are less sensitive to cyclosporin A-induced nephrotoxicity than humans. In the present study, typical functional disturbances, such as a decrease in creatinine clearance, were observed in cyclosporin A-treated SHR. An increase in fractional excretion of sodium, an index of tubular function, was also observed in cyclosporin A-treated rats, indicating impaired tubulointerstitial function. From histological analysis, typical morphological changes, such as proximal tubular vacuolization, were observed in cyclosporin A-treated rats. An important new finding in this study was that all of the functional and morphological changes were improved by administration of FR226807. In our preliminary study, the restoration of renal function was also observed with sildenafil, a clinically used phosphodiesterase type 5 inhibitor, suggesting that the beneficial effect is not a FR226807 specific effect (data not shown).

Phosphodiesterases, as well as guanylate cyclase, are key factors for controlling cellular cyclic nucleotides. Phosphodiesterase activities are regulated by multiple input from other signaling systems and are a crucial point of integration of intracellular regulatory networks (Houslay, 1995, Houslay and Milligan, 1997; Dousa, 1998). The phosphodiesterase activities are crucial for cellular signaling, as metabolism of cyclic nucleotides modulates their intracellular concentration and affects subsequent cellular and behavioral responses. The role of phosphodiesterases in mediating cyclosporin A nephrotoxicity, however, has not been characterized. Recently, we found an increase in cGMP content in kidney, probably due to the combination of increase in guanylate cyclase activity and decrease in phosphodiesterase activity, despite the marked decrease in NOS activity from cyclosporin A nephrotoxicity (Hosogai et al., 2001a). We suggested that this reciprocal mechanism is probably maintaining homeostasis in the kidney treated with cyclosporin A (Hosogai et al., 2001a), but not enough to prevent progression of cyclosporin A nephrotoxicity.

The exact mechanism by which FR226807 protects the kidney from cyclosporin A-induced nephrotoxicity is not clear from our current data and further studies are necessary to clarify this issue. However, it is likely that the protective effect of FR226807 is derived from the increase in cGMP content causing further inhibition of phosphodiesterase type 5. This is also supported by the following experimental evidence, i.e., no cGMP-phosphodiesterase activity was detected in the kidney isolated from FR226807-treated animals, and FR226807 itself has no effect on iNOS, cNOS or guanylate cyclase activity in the kidney, in this study.

Major protective effects of FR226807 were associated with inhibition of residual cGMP-phosphodiesterase activity. However, about a 90% decrease in activity by cyclosporin A itself was unable to prevent occurrence of nephrotoxicity on the 15th day. It is probable that a decrease in NO synthesis and nephrotoxicity are the primary events in cyclosporin A-treated kidney. Because down-regulation of cGMP-phosphodiesterase activity may be a slowly occurring response to compensate decreased NO, nephrotoxicity was not sufficiently prevented for its low activity at 15 days. As FR226807 is a quick-onset inhibitor of cGMP-phosphodiesterase, cGMP-phosphodiesterase activity may be kept low throughout the experiment and cyclosporin A nephrotoxicity may be prevented. Nevertheless, FR226807 could not sufficiently prevent dysfunction, suggesting that a number of mechanisms other than cGMP may also be involved in the development of cyclosporin A nephrotoxicity.

There are reports that antihypertensive drugs, such as angiotensin-converting enzyme inhibitor enalapril or the angiotensin II type 1 receptor antagonist valsartan, attenuated cyclosporin A-induced hypertension and considerably prevented renal toxicity in rats (Lassila et al., 2000). However, the protective effect of FR226807 may not be due to the antihypertensive effect, as only modest effects of phosphodiesterase type 5 inhibitor on blood pressure have been observed (Vallance, 1996). Although we have no data relating to blood pressure associated with use of FR226807 in SHR, there are reports showing that selective phosphodiesterase type 5 inhibitors only slightly affect blood pressure. For example, sildenafil only slightly reduced blood pressures in hypertensive men (Vardi et al., 2002). E4021 did not affect either blood pressure or heart rate in SHR even in excessive doses (Vemulapalli et al., 1996). These results are in agreement with our preliminary study, that both sildenafil and our selective phosphodiesterase type 5 inhibitors (derivatives of FR226807) showed only modest hypotensive effects (about 10% decrease) when orally administered in spontaneous hypertensive rats. Therefore, we hypothesize that FR226807 may affect cyclosporin A nephrotoxicity via elevated cGMP content, rather than via reducing blood pressure.

Other mediators that might contribute to cyclosporin A nephrotoxicity have been suggested, including the free radical, Ca^{2+}, extracellular matrix and renin–angiotensin systems. cGMP might antagonize these candidates for mediators of cyclosporin A nephrotoxicity. It has been reported that cyclosporin A increases free radical formation, which appears important in the pathogenesis of cyclosporin A nephrotoxicity (Wang and Salahudeen, 1995; Zhong et al., 1998). As the cGMP-dependent pathway increases antioxidant heme oxygenase-1 protein levels, increases in

cGMP are expected to produce renal protection and attenuate cyclosporin A toxicity (Polte et al., 2002). There is evidence that administration of cyclosporin A causes the accumulation of intracellular Ca^{2+} in smooth muscle via influx through voltage-gated Ca^{2+} channels (Kristian and Siesjo, 1998). The rise of intracellular Ca^{2+} may lead to contraction and necrosis in vascular smooth muscle (Pere et al., 1998). cGMP lowers cytosolic Ca^{2+} concentration in smooth muscle and various other cell types, and cGMP content was comparable to that during smooth muscle relaxation (Ruth, 1999). Another action of cyclosporin A is the accumulation of extracellular matrix molecules, with experimental and clinical studies implicating a role for transforming growth factor-beta (TGF-β) in the effect of cyclosporin A on matrix regulation (Islam et al., 2001). There is increasing evidence that NO can negatively regulate extracellular matrix protein expression (Kolpakov et al., 1995; Hou et al., 1995). Additionally, in vitro studies have demonstrated that thrombospondin 1-dependent TGF-β bioactivity is down-regulated by a NO-cGMP-dependent mechanism (Wang et al., 2002). These lines of evidence suggest that cGMP is likely to have some protective effect for impaired renal function induced by cyclosporin A via a variety of mechanisms. However, it is still difficult to assess which of these properties is responsible for the protective effect induced by FR226807.

In conclusion, we have shown that FR226807, a selective phosphodiesterase type 5 inhibitor, ameliorates cyclosporin A nephrotoxicity with further increases in cGMP content. Modulators of the NO-cGMP pathway, causing an increase in cGMP content in the kidney, are a potential pharmacological target for treatment of cyclosporin A nephrotoxicity.

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