Inhibition of nuclear factor-κB activation by pyrrolidine dithiocarbamate prevents chronic FK506 nephropathy

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Inhibition of nuclear factor-κB activation by pyrrolidine dithiocarbamate prevents chronic FK506 nephropathy.

Background. Chronic tacrolimus (FK506) nephrotoxicity is characterized by renal fibrosis with interstitial inflammation. Since nuclear factor-κB (NF-κB) plays a key role in chronic inflammatory diseases including renal disease, the present study was conducted to elucidate the role of NF-κB in the pathogenesis of chronic FK506-induced nephropathy.

Methods. FK506 (1 mg/kg/day, SC) was administered daily to rats maintained on low sodium diet for 42 days. Some rats were treated with a putative NF-κB inhibitor, pyrrolidine dithiocarbamate (PDTC; 100, 200 mg/kg/day, by gavage). The renal function, renal histology, renal NF-κB–DNA binding activity and gene expression profile were examined.

Results. FK506 caused a decline in glomerular filtration and induced characteristic renal morphologic changes including arteriolopathy, tubular atrophy and interstitial fibrosis. FK506 markedly activated renal cortical NF-κB–DNA binding. PDTC administration inhibited NF-κB–DNA binding activity in a dose dependent manner. With higher dose, NF-κB–DNA binding activity was decreased to a control level. PDTC had little effect on FK506-induced renal dysfunction. Renal cortical monocyte/macrophage infiltration observed in FK506-treated rats was dramatically suppressed by PDTC. FK506 up-regulated renal cortical gene expression of chemokine receptors, monocyte chemoattractant protein-1 (MCP-1) and osteopontin. PDTC significantly blocked MCP-1 gene expression but had no effect on osteopontin gene expression. Tubular atrophy and tubulointerstitial fibrosis, but not arteriolopathy, were significantly attenuated by PDTC. FK506 increased renal mRNA expression of fibrogenic molecules and extracellular matrices that also were attenuated by PDTC treatment.

Conclusions. NF-κB plays an important role in mediating cortical monocyte/macrophage infiltration and in the pathogenesis of tubular injury and interstitial fibrosis in experimental FK506-induced chronic nephropathy.

Tacrolimus (FK506) is a potent immunosuppressive agent that is effective in allograft prophylaxis after organ transplantation. However, its clinical use is limited by nephrotoxicity. Acute nephrotoxicity is a hemodynamic change characterized by renal vasoconstriction that is dose-related and reversible. On the other hand, chronic FK506-induced nephrotoxicity is thought to be progressive and irreversible. These changes include tubular atrophy, afferent arteriolar hyalinosis and striped interstitial fibrosis with mononuclear cell infiltration. The precise mechanisms of FK506-induced nephrotoxicity are not completely understood.

In cyclosporine A (CsA) nephropathy, the inflammation accompanied with macrophage infiltration precedes interstitial fibrosis [1, 2]. Precedence of such inflammatory responses leads to the development of various types of tubulointerstitial diseases [3–5]. Furthermore, such inflammatory cells are a known source of several fibrogenic molecules [6].

Nuclear factor-κB (NF-κB) plays a central role in many chronic inflammatory diseases [7] including renal disease [8]. NF-κB transcriptionally regulates a variety of cellular genes, including pro-inflammatory cytokines, enzymes and adhesion molecules in chronic inflammatory diseases [7]. Among them, monocyte chemoattractant protein-1 (MCP-1) is a potent macrophage chemoattractant [9], the expression of which is regulated by NF-κB [10]. We previously reported that MCP-1 mRNA expression correlate with monocyte/macrophage infiltration in rat model of chronic CsA nephropathy [2].

This study was designed to test the hypothesis that activation of NF-κB in the renal cortex mediates the monocyte/macrophage influx into the renal cortical inte-
stitium and leads to tubulointerstitial fibrosis in chronic FK506 nephrotoxicity. The first aim of our study was to establish whether NF-κB is activated in the renal cortex of rats with chronic FK506 nephrotoxicity. The second aim was to evaluate the effect of NF-κB inhibition on monocytic/macrophage infiltration, tubular injury and striped interstitial fibrosis seen in chronic FK506 nephrotoxicity.

**METHODS**

**Animals**

The present experiments were conducted in accord with NIH Guide for Care and Use of Laboratory Animals. All rats and diets were purchased from Clea Japan (Tokyo, Japan). Adult male Sprague-Dawley rats weighing 180 to 200 g at the onset of the experiments were housed three to a cage and were allowed free access to standard rat chow for three days in a temperature and light controlled environment. Rats were then housed in individual cages and received a low sodium diet (0.05% sodium) for a week before FK506 administration.

**Drugs**

FK506 and vehicle [mannitol 2.5 mg/mL and HCO60 (poloxoyethylated hydrogenated castor oil) 0.2 mg/mL] were a gift from Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). FK506 was suspended in its vehicle at a final concentration of 1 mg/mL. Pyrrolidine dithiocarbamate (PDTC; Sigma-Aldrich, Tokyo, Japan) was dissolved in distilled water to a final concentration of 100 and 200 mg/mL.

**Experimental groups**

During the entire experimental period the rats were maintained on a low sodium diet. Since FK506 treatment decreased food consumption, pair-feeding was performed in vehicle-treated rats. Body weight was monitored daily. To examine the effect of NF-κB inhibition, rats were divided into four groups as follows:

1. FK506. Rats received a daily SC injection of FK506 (1 mg/kg/day) for 42 days ($N = 10$);
2. FK506 + PDTC 100 mg. Rats received a daily SC injection of FK506 (1 mg/kg/day) for 42 days and received PDTC 100 mg/kg/day by gavage ($N = 8$);
3. FK506 + PDTC 200 mg. Rats received a daily SC injection of FK506 (1 mg/kg/day) for 42 days and received PDTC 200 mg/kg/day by gavage ($N = 8$); and
4. Vehicle. Rats received a daily SC injection of vehicle (1 mL/kg/day) for 42 days ($N = 10$).

**Experimental protocol**

Following the last dosing, rats were housed in individual metabolic cages for 24-hour urine collection. At the end of the study, animals were anesthetized with sodium pentobarbital (50 mg/kg, IP) and a ventral midline incision was made. After blood sampling from aorta, both kidneys were perfused with 20 mL cold heparinized saline. Kidneys were immediately excised and the cortices were carefully dissected from the medulla, snap frozen in liquid nitrogen and stored at −80°C until use for RNA extraction. A portion of renal cortical tissue was processed for isolation of nuclear protein. The remaining renal tissues were fixed in 4% paraformaldehyde or methyl Carnoy’s solution.

**Functional studies**

Plasma and urinary creatinine and electrolytes were measured by standard laboratory methods. The whole blood FK506 level was determined using commercially available enzyme-linked immunosorbent assay (ELISA) kit. Blood pressure was measured by tail-cuff method.

**Morphology**

Three-micron thick sections of paraformaldehyde-fixed renal tissue were stained with periodic acid-Schiff’s reagent (PAS). Tissue samples were evaluated for tubular injury, interstitial fibrosis and arteriolopathy by an observer masked to the treatment groups.

Tubular injury was scored semiquantitatively for at least 30 cortical fields ($\times 100$ magnification) of PAS stained sample [11]. Tubular injury was defined as tubular dilation, atrophy, and thickening of tubular basement membrane. The following semiquantitative score was used: Score 0 = no tubular injury; Score 1 = <10% of the tubules were injured; Score 2 = 10 to 25% of the tubules were injured; Score 3 = 25 to 50% of the tubules were injured; Score 4 = 50 to 75% tubules injured; Score 5 = >75% of tubules injured.

Interstitial fibrosis was scored semiquantitatively at least 30 cortical fields ($\times 200$ magnification) of PAS stained sample. The following semiquantitative score was used: Score 0 = normal interstitium and tubules; Score 1 = mild fibrosis in the tubulointerstitium; Score 2 = modest fibrosis with moderate interstitial widening between tubules; Score 3 = severe fibrosis with severe interstitial widening between the tubules.

Arteriolopathy was semiquantitatively estimated by counting the percentage of juxtaglomerular arterioles with hyalinosis per total number of juxtaglomerular arterioles, with a minimum of 60 glomeruli per tissue sample assessed.

**Preparation of renal cortex nuclear protein extracts**

Nuclear protein extractions were performed as described previously with minor modification [12]. Briefly, cortex tissue from each rat was rapidly suspended in 0.4 mL of 10 mmol/L Hepes, pH 7.9, containing 10 mmol/L KCl, 0.1 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.1 mmol/L ethyleneglycol-bis-(β-aminoethyl ether)-N,N′-
The sequence of double-stranded oligonucleotide used for EMSA was as follows: consensus NF-κB, 5′-AGT TGA GGG GAC TTT CCC AGG C-3′ (Promega, Madison, WI, USA). This probe was end-labeled with [γ-32P] adenosine 5′-triphosphate (ATP), using T4 polynucleotide kinase and purified by chromatography on a Bio-Spin column (Bio-Rad, Richmond, CA, USA). The nuclear protein samples (5 μg of protein) were incubated with 32P-labeled consensus oligonucleotide for 20 minutes at room temperature in 20 mmol/L Hepes, pH 7.9, 0.3 mmol/L EDTA, 0.2 mmol/L EGTA, 80 mmol/L NaCl, 1 mmol/L DTT, 0.2 mmol/L PMSF, 6% glycerol and 2 μg of poly[dI-dC]. The DNA-protein complexes were separated from free DNA probe by electrophoresis on 6% non-denaturing acrylamide gels in 6.7 mmol/L Tris-HCl, pH 7.5, 3.3 mmol/L sodium acetate, 0.1 mmol/L EDTA and 2.5% glycerol. Gels were run at 200 V in a cold room (4°C) for three hours, dried, and contacted overnight with imaging plate. Specific band was analyzed with bioimaging analyzer (BAS 2500; Fuji Photo Film Co., Tokyo, Japan). To demonstrate the specificity of DNA-protein binding, the binding reactions were performed in the presence of nonlabeled consensus oligonucleotide competitor. Furthermore, a supershift assay was carried out using rabbit polyclonal IgG against p50, p52 and p65 (Santa Cruz Technology, Santa Cruz, CA, USA). For the supershift assay, the binding reaction of nuclear protein with the labeled oligonucleotide was carried out in the presence of specific antibodies, followed by electrophoresis.

**Immunohistochemistry**

Three-micron thick sections of renal tissue fixed with methyl Carnoy’s solution were stained with ED-1 antibody by labeled streptavidin-biotin (LSAB) method. After deparaffinization and rehydration, endogenous peroxidase activity was quenched by 3% H2O2 for 10 minutes and the sections were contacted with 5% fetal calf serum for 30 minutes. Next, sections were incubated with mouse monoclonal antibody to the rat monocyte/macrophage (ED-1, 1:500 dilution; Serotec, Oxford, UK) for one hour at room temperature. Biotinylated anti-mouse immunoglobulin, horseradish peroxidase-conjugated streptavidin (Dako LSAB2; Dako Corp., Carpenteria, CA, USA) were used according to manufacturer’s instructions. Immunoreaction products were developed using 3,3′-diaminobenzidine (DAB) as the chromogen. Sections were then counterstained with methyl green. Monocyte/macrophage infiltration was quantified by counting the number of ED-1 positive cells in 20 randomly chosen 1.0 × 1.0 mm areas of renal cortex.

**mRNA**

RNA extracted by guanidium thiocyanate-phenol-chloroform method as previously reported [11] was used for Northern blot analysis. Then, 10 μg total RNA was электрофоресed on 1.2% agarose gel containing 2.2 mol/L formaldehyde and 0.2 mol/L MOPS (pH 7.0), and transferred to a nylon membrane by using the Turboblotter Rapid Downward Transfer System (Schleicher & Schuell, Dassel, Germany). The nucleic acids were crosslinked by ultra-

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<th>Parameters</th>
<th>Treatment</th>
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<tr>
<td>Body weight gain g</td>
<td>FK506 125 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>FP100 51 ± 18&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>FP200 19 ± 8&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>VH 178 ± 6</td>
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<td>Systolic blood pressure ( \text{mm Hg} )</td>
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<tr>
<td></td>
<td>FP100 122 ± 1</td>
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<td></td>
<td>FP200 119 ± 1</td>
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<tr>
<td></td>
<td>VH 125 ± 1</td>
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<tr>
<td>Plasma creatinine concentration ( \text{mg/dL} )</td>
<td>FK506 0.92 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>FP100 0.70 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>FP200 0.87 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>VH 0.28 ± 0.01</td>
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<td>Creatinine clearance ( \text{mL/min} )</td>
<td>FK506 1.477 ± 0.124&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>FP100 1.964 ± 0.129&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>FP200 1.796 ± 0.204&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>VH 2.749 ± 0.139</td>
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<td>Creatinine clearance ( \text{mL/min/100 g body weight} )</td>
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<td>FP200 0.590 ± 0.069</td>
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<td>FP100 14.1 ± 0.6</td>
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<td>FP200 13.6 ± 1.1</td>
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<sup>a</sup>Values are means ± SE. Abbreviations are: FP100, FK506 with PDTC (100 mg/kg/day); FP200, FK506 with PDTC (200 mg/kg/day); VH, vehicle. FK506 (N = 10), FP100 (N = 8), FP200 (N = 8), VH (N = 10).

<sup>b</sup>P < 0.05 vs. vehicle
<sup>c</sup>P < 0.05 vs. FK506
<sup>d</sup>P < 0.05 vs. FP100
violet irradiation (Stratagene, La Jolla, CA, USA). The membranes were prehybridized at 68°C for an hour with QuickHyb hybridization solution (Stratagene). The membranes were hybridized at 68°C overnight with 32P-dCTP-labeled cDNA probes, washed, and finally exposed to imaging plate. The specific bands were analyzed with the bioimaging analyzer (BAS-2500). For all of the RNA samples, density of an individual mRNA band was divided by that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA band to correct for the difference in RNA loading and/or transfer.

The cDNA probes used were rat MCP-1 cDNA and rat osteopontin cDNA [2]. Other cDNAs used were rat transforming growth factor-β1 (TGF-β1), mouse plasminogen activator inhibitor 1 (PAI-1), rat tissue inhibitor of metalloproteinase inhibitor 1 (TIMP-1), rat α1 (type 1) collagen, mouse α1 (type IV) collagen and rat GAPDH, all of which have been described previously [11].

**Statistical analysis**

Data were presented as mean ± SE. All data were analyzed by using analysis of variance (ANOVA) and individual comparisons were made by using Duncan’s multiple range test. Statistical significance was defined as \( P < 0.05 \).

**RESULTS**

**Physiologic study**

Physiologic parameters are summarized in Table 1. The final body weight gain in FK506 group was lower than that of vehicle group despite of pair-feeding. Further, animals treated with PDTC failed to gain as much weight as those receiving FK506 alone. FK506 did not increase blood pressure in this model and PDTC did not affect it.

Plasma creatinine in the FK506-treated group was significantly higher than that of vehicle group. PDTC at lower dose slightly but significantly attenuated the FK506-induced increase in plasma creatinine concentration, whereas at high dose PDTC was without effects. As PDTC treatment retarded body weight gain, endogenous creatinine clearance (C\(_{\text{Cr}}\)) was calculated per animal and per body weight basis and were both shown in Table 1. FK506 significantly decreased C\(_{\text{Cr}}\). Although PDTC appeared to attenuate FK506-induced reduction in C\(_{\text{Cr}}\), such effects were marginal.

**Histologic change**

FK506 treated rats had characteristic morphologic lesions that were similar to the human chronic nephropathy observed after long-term treatments with CsA and FK506. Figure 1 shows a typical example of the morphology by microphotography and Figure 2 summarizes the morphologic data of all groups. FK506 treatment caused a prominent injury consisting of tubular dilation, tubular atrophy, sloughing of tubular epithelial cells and thickening of the tubular basement membrane. PDTC dose-dependently reduced the degree of tubular injury. Rats treated with FK506 developed a striped interstitial fibrosis that was attenuated also by PDTC. Arteriolopathy of the afferent arteriole was present in FK506-treated rats but it was not attenuated by PDTC treatment.

**Electrophoretic mobility shift assay**

Renal cortical NF-κB-DNA binding activity was significantly increased by FK506 (Fig. 3). The composition of FK506-activated NF-κB complex consisted predomi-
Fig. 3. Renal cortical NF-κB binding in different groups by electrophoretic mobility shift assay (EMSA). Abbreviations are in the legend to Figure 2. (A) Autoradiogram of EMSA. The bracket indicates specific NF-κB-DNA binding. Competition assay for NF-κB were carried out in the presence of 50-fold molar excess unlabeled NF-κB probe (×50) are shown also. Supershift bands with p50 and p65 antibodies were observed whereas p52 antibody did not shift the band. (B) Quantification of the density of each autoradiogram. Results are expressed as mean ± SE. *P < 0.05 vs. vehicle, †P < 0.05 vs. FK506, #P < 0.05 vs. FP100.

Immunohistochemistry

Monocyte/macrophage infiltration was increased by FK506 (Fig. 4). It was predominantly detected in the widened interstitium around injured tubules. Monocyte/macrophage influx was dramatically attenuated by PDTC in a dose-dependent manner. Especially in the PDTC 200 PDTC dose-dependently attenuated the FK506-induced increase in NF-κB-DNA binding. With a higher dose of PDTC, NF-κB-DNA binding activity was decreased to a level not different from that of vehicle-treated rats.
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with calcineurin inhibitors, CsA and FK506. PDTC almost completely blocked the FK506-induced increase in NF-κB binding activity and monocyte/macrophage influx. PDTC also attenuated the FK506-induced tubular injury and interstitial fibrosis. Thus, NF-κB activation could play an important role in the progression of renal injury in chronic FK506 nephropathy.

Activation of NF-κB transcription factor family plays a central role in various chronic inflammatory diseases [7], including renal disease [8], through its ability to induce transcription of proinflammatory genes [13]. The association of tubulointerstitial inflammation with increased NF-κB–DNA binding activity implies a role for NF-κB in the pathogenesis of chronic FK506 nephropathy. Furthermore, PDTC treatment dramatically blocked the activation of NF-κB–DNA binding and interstitial infiltration of monocyte/macrophage. Both in vivo and in vitro evidence demonstrate that PDTC is a putative inhibitor of NF-κB [14, 15]. Although the precise mechanism of the effect of PDTC on NF-κB sequestrations is unknown, the present findings support the notion that NF-κB activation is implicated in the FK506-induced influx of mononuclear cells.

Infiltration of mononuclear cells into the cortical interstitium was associated with up-regulation of MCP-1, a potent macrophage chemoattractant [9]. We previously showed an increased expression of MCP-1 in rat model of chronic CsA nephrotoxicity [2]. It is well known that NF-κB plays an important role in the transcriptional control of MCP-1 [10]. As anticipated, PDTC attenuated MCP-1 gene expression, but the effect was partial. Thus, inhibition of MCP-1 expression alone could not explain the almost complete blockade of macrophage infiltration by PDTC. It is possible that additional cytokines, enzymes or adhesion molecules regulated by NF-κB also were involved in the FK506-induced macrophage influx. It should be pointed out that osteopontin is another macrophage chemoattractant [16] that was markedly up-regulated with FK506 treatment. However, PDTC had no effect on osteopontin expression, thus eliminating the role of osteopontin in inducing the macrophage influx in chronic FK506 nephrotoxicity.

Inflammatory responses such as monocyte/macrophage influx are common features that lead to the development of various types of tubulointerstitial disease [3–5]. In CsA nephropathy, the interstitial inflammation accompanied by macrophage infiltration preceded interstitial fibrosis [1, 2]. Monocyte/macrophage is a known source of several fibrogenic genes including TGF-β, which is highly correlated to fibrogenic processes [6]. These notions together with the present finding that PDTC blocked FK506-induced macrophage influx and attenuated tubular injury and tubulointerstitial fibrosis suggest that blockade of mononuclear cell infiltration and inflammation contributed to the attenuation of FK506-induced renal fibrosis.

**DISCUSSION**

The present study shows that long-term treatment of rats with FK506 elicits chronic nephropathy that is associated with an increase in renal cortical NF-κB–DNA binding activity. These lesions include arteriolopathy, tubular injury (tubular dilation, tubular atrophy and thickening of tubular basement membrane), interstitial monocyte/macrophage influx and striped interstitial fibrosis, which mimics chronic nephropathy seen in patients treated

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**Fig. 4. Effects of PDTC treatment on renal cortical infiltration of monocyte/macrophage.** Abbreviations are in the legend to Figure 2. Monocyte/macrophage infiltration was evaluated by ED-1 immunostaining. (A) FK506-treated rats showed a marked influx of ED-1 positive cells that was predominantly detected in widened interstitium around injured tubules at day 42. (B) This influx was nearly abolished with PDTC (200 mg/kg/day; magnification ×40). (C) Semiquantitative scoring analysis of ED-1 positive cells. Results are expressed as mean ± SE. *P < 0.05 vs. vehicle; †P < 0.05 vs. FK506; #P < 0.05 vs. FP100.
Fig. 5. Effects of PDTC treatment on renal cortical mRNA in FK506-treated rats. Abbreviations are in the legend to Figure 2. Symbols are: (■) FK506; (□) FP100; (▲) FP200; (△) VH. Ten micrograms of total RNA was isolated from cortex and hybridized with cDNA probe to MCP-1, osteopontin, TGF-β1, collagen I and IV, PAI-1 and TIMP-1. The ordinate shows each mRNA values corrected for GAPDH mRNA values. Results are expressed as mean ± SE. *P < 0.05 vs. vehicle, †P < 0.05 vs. FK506, #P < 0.05 vs. FP100.
with PDTC treatment. Such a histologic improvement was supported by the finding that PDTC attenuated FK506-induced gene expressions of pro-fibrogenic molecules such as TGF-β, PAI-1 and TIMP-1 and extracellular matrix component, collagens I and IV. TGF-β directly stimulates extracellular matrix including collagens I and IV [6], and also blocks extracellular matrix degradation by stimulating protease inhibitor, like PAI-1 and TIMP-1 [17]. Shihab et al showed that TGF-β is involved in FK506-induced nephropathy [18].

It should be pointed out, however, that an almost complete blockade of macrophage influx and NF-κB-DNA binding is in contrast with the partial inhibition of renal fibrosis and corresponding attenuation of gene expression directly related to renal fibrosis. Thus, it is possible that NF-κB-independent mechanisms also are likely to play a role in the pathogenesis in chronic FK506-induced interstitial fibrosis.

The common belief is that calcineurin inhibitors block NF-κB activity in lymphoid cells [19, 20]. Even in non-lymphoid cells CsA inhibits the NF-κB activity of rat mesangial cells [21], and FK506 also inhibits NF-κB activity of rat hepatocytes [22]. Although the mechanism by which FK506 treatment activates renal cortical NF-κB-DNA binding remains to be elucidated, angiotensin II is one of possible candidates that activated renal NF-κB activity [23]. It has been well recognized that the renin-angiotensin system is involved in chronic FK506 nephrotoxicity [24] and CsA nephrotoxicity [25, 26]. Renal cortical expression of renin mRNA is up-regulated with FK506 treatment [18, 27]. Another possible candidate that plays a central role in NF-κB activation is reactive oxygen species (ROS) [28]. Some reports suggest that oxidative stress may contribute to the pathogenesis of chronic CsA nephrotoxicity [29, 30]. Finally, it should be pointed out that FK506 directly activates NF-κB in non-lymphoid cells through degradation of I-κBα [31]. Thus, such a mechanism may be operating also in this phenomenon.

In the present study, PDTC did not affect FK506-induced arteriolopathy despite the beneficial effects on macrophage infiltration and fibrotic lesions, suggesting that arteriolar lesions were unrelated to NF-κB activation. Furthermore, as the effects of PDTC on glomerular dysfunction were marginal, it is unlikely that NF-κB-dependent mechanisms were mainly operating in FK506-induced renal dysfunction.

The dithiocarbamates, such as PDTC, represent a class of antioxidants reported to be a potent inhibitor of NF-κB [32]. The most effective inhibitor of NF-κB may be PDTC as a result of its ability to transverse into the cell and prolonged stability in solution at physiological pH [33] and its ability to suppress the production of ROS [34]. However, PDTC-treated rats showed a retarded body weight gain, suggesting the presence of a toxic action of PDTC. Despite such toxicities, PDTC successfully blocked the activation of NF-κB induced by FK506.

In summary, the present study clearly shows that in vivo activation of NF-κB plays an important role in the progression of chronic FK506 nephropathy. Therefore, clinical study about how chronic FK506 nephrotoxicity blocks NF-κB activation may prove worthwhile. Further studies are needed to elucidate the role of NF-κB in FK506-induced nephropathy. For this purpose, the localization of the activated NF-κB proteins should be clarified. Such a study should be followed by experiments using more specific NF-κB inhibitions such as NF-κB decoy oligodeoxynucleotide or dominant negative I-κB.

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REFERENCES


