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The Effect of Immunosuppressive Agents (FK-506, Rapamycin) on Renal P450 Systems in Rat Models

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Abstract

It is well known that cyclosporin, rapamycin and FK-506 (tacrolimus) are metabolized by the liver microsomal cytochrome P450 enzyme system. Although there have been reports of interaction between these drugs and the renal P450 enzyme system, differences among these immunosuppressants has not been comprehensively demonstrated. We have studied the individual capacities of these immunosuppressants to induce renal microsomal P450 enzymes similar to CYP2B4 and CYP4A2 by examining renal function in treated rats, and have correlated the results by means of biochemical, immunological and immunohistochemical assays of renal P450 enzymes.

Cyclosporin caused impairment of renal function with an increase in renal-specific P450 content, but FK-506 and rapamycin did not. Laurate ω- and (ω-1)-hydroxylase activity increased in rats treated with rapamycin but decreased in those treated with FK-506. Prostaglandin A_1 (PGA_1) ω-hydroxylase activity increased in rats treated with FK-506 but was reduced by treatment with cyclosporin. Aminopyrine *N*-demethylase activity increased in rats treated with cyclosporin or FK-506, but not in those treated with rapamycin. Western-blot analysis revealed significant induction of P450, (similar to CYP2B4 of the rabbit P450 isozyme) in kidneys from rats treated with cyclosporin but not in those from rats receiving FK-506 or rapamycin. Histochemical studies clearly demonstrated a form of P450 such as CYP4A2 in the proximal tubules of rats treated with cyclosporin, but not in those of rats treated with FK-506 or rapamycin.

These results show that although cyclosporin has a strong effect on renal P450 systems and induces such a system in kidney cortex (microsomal P450), FK-506 and rapamycin have no substantial effect on the induction of renal P450. These findings might clarify the nephrotoxicity induced by these immunosuppressive drugs.

Although cyclosporin is widely used in clinical transplantation (Calen 1987; Solders et al 1987; Hakim et al 1988; Iwatsuki 1988) it has several side effects (Kahan 1989), the most serious of which is nephrotoxicity. Possible mechanisms of cyclosporin-induced nephrotoxicity include vasoconstriction of afferent glomerular arterioles (English et al 1987), cyclosporin-induced release of catecholamines (Marray et al 1985), cyclosporin-potentiated vasoconstrictor hormone-induced, transmembrane calcium ion influx (Goldberg et al 1989) and alteration of the balance of the vasodilator prostacyclin and its vasoconstrictor antagonist thromboxane A_2 (TxA_2) in renal cortical tissue (Coffman et al 1987). Much attention has been focused on the effect of cyclosporin on prostanoid metabolism, because a shift in balance away from the vasodilatory prostaglandins and toward the vasoconstrictory prostaglandins and thromboxanes could promote vasoconstriction. Although it is generally believed that a specific liver P450 enzyme (CYP3A4 in man) catalyses the hydroxylation and demethylation of cyclosporin at different sites, to produce a variety of metabolites (Bertault-Peres et al 1987; Watkins 1990; Fromm et al 1996; Gan et al 1996; Pichard et al 1996; Watkins 1996; Zhou et al 1996), little is known about the role of the kidney microsomal P450 systems in the

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metabolism of cyclosporin. Our previous studies have revealed that cyclosporin induces renal microsomal P450 enzymes in rats and there is a direct correlation between the induction of P450 in the kidney and its functional impairment (Yoshimura et al 1989).

It has recently been reported that the immunosuppressive activity of rapamycin and FK-506 are significantly higher than that of cyclosporin, and that rapamycin and FK-506 result in a lower incidence of nephrotoxicity (Kino et al 1987; Ochiai et al 1987; Todo et al 1987). It is also generally accepted that these drugs are metabolized by the liver P450 system (CYP3A4 in man), as is cyclosporin, although few reports about the relationship between these two drugs and the renal P450 enzyme systems have been published (Krueger et al 1991; Vincent et al 1991). The purpose of this study was to assess the individual capacities of these immunosuppressants to induce P450 enzymes similar to CYP2B4 and CYP4A2 in kidney tissue, and to assess the metabolism of the tested substrates by renal microsomes isolated from rats treated with these agents.

Materials and Methods

Animals and treatment

Experiments were performed on Male Lewis rats, 150–200 g. Group A (n = 5) was an untreated control group; group B (n = 5) received olive oil alone (0·1 mL daily); group C (n = 5) received cyclosporin (30 mg kg^{-1}/day) dissolved in olive oil (0·1 mL); group D (n = 5) received FK-506 (1 mg kg^{-1}/day) dissolved in olive oil (0·1 mL); group E (n = 5) received FK-506 (3 mg kg^{-1}/day) dissolved in olive oil (0·1 mL); group F (n = 5) received vehicle alone (10% Tween 80, 20% *N*, *N*-dimethylacetamide, and 70% polyethylene glycol 400; 0·1 mL); group G (n = 5) received rapamycin (0·3 mg kg^{-1}/day) dissolved in vehicle (0·1 mL); group H (n = 5) received rapamycin (3 mg kg^{-1}/day) dissolved in vehicle (0·1 mL). Rats in groups B, C, D, E, F, G and H were treated for 14 days by intramuscular injection. Blood samples were drawn from the tail veins of rats in each group before treatment, and on days 7 and 14 after initiation of treatment. Statistical evaluation was performed by use of Student's *t*-test.

Preparation

After treatment of rats with cyclosporin, FK-506 or rapamycin for 14 days kidneys were homogenized in 0·25 M sucrose, 20 mM potassium phosphate buffer (pH 7·5), and 1 mM EDTA for 1 min. The homogenates were centrifuged at 2700 *g* for 15 min and the resulting supernatant was then centrifuged at 11 000 *g* for 15 min. The supernatant resulting from this second centrifugation was then centrifuged at 78 000 *g* for 60 min. The sediment was washed once and used as a microsomal preparation. CYP2B4 was prepared, by the method of Imai et al (1980), from liver microsomes of rabbits treated with phenobarbital. Antibodies against CYP2B4 were prepared from guinea-pigs as described elsewhere (Yamamoto et al 1986).

Biochemical methods

P450 content was measured by the method of Omura & Sato (1964) from the CO-difference spectrum of a dithionite-reduced sample; a value of 91 cm^{-1} mM^{-1} cm was used for the extinction coefficient between 450 and 490 nm. Protein concentrations were measured by the procedure of Lowry et al (1951). To determine laurate ω- and (ω-1)-hydroxylase activity, microsomes (0·15 mg protein) were incubated with Tris–HCl buffer (pH 7·5, 30 μmol), NADPH (70 nmol) and [1-^{14}C]laurate (7×10^4 counts min^{-1}, 10·9 nmol), in a total volume of 0·3 mL at 37°C for 10 min. The combined ω- and (ω-1)-hydroxylation of laurate was determined as described elsewhere (Kusunose et al 1981). To determine prostaglandin A_1 (PGA_1) ω-hydroxylase activity, microsomes (0·6 mg protein) were incubated with sodium phosphate buffer (pH 7·4, 30 μmol), glucose-6-phosphate (12 μmol), glucose-6-phosphate dehydrogenase (0·9 units), NADP (0·4 μmol), and PGA_1 (40 nmol) in a total volume of 0·3 mL at 37°C for 10 min. ω-Hydroxy PGA_1 was determined as described elsewhere (Kusunose et al 1984). Aminopyrine *N*-demethylase activity was determined by the method of Nash (1953).

Western-blot analysis

Western blotting of proteins was resolved by sodium dodecyl sulphate (SDS) gel electrophoresis on nitrocellulose paper, then immunochemical identification using a second antibody directed against guinea-pig IgG linked to peroxidase was performed.

Histochemical methods

Kidney sections (30 μm) were cut on a cryostat (Bright, 5030 microtome), rinsed three times in 0·1 M phosphate-buffered saline (PBS), and

immersed ''free-floating'' in PBS containing 10% normal goat serum for 2 h. The sections were placed in a small vial and incubated with guinea-pig antibodies against CYP4A2 for 48 h at 4°C (Kawashima et al 1992). The IgG form of the antibody was obtained from rabbits immunized with purified CYP4A2 (P450K2). The sections were then washed three times with PBS and incubated overnight at 4°C with a 1 : 500 dilution of fluorescent isothiocyanate-labelled goat anti-rabbit IgG (VECTOR rabs). After rinsing with PBS the sections were mounted on glass slides in glycerine-PBS (1 : 1) and analysed under a confocal microscope (Olympus LSM-GB 200) equipped with a B-dichroic filter system for measurement of fluorescent isothiocyanate fluorescence.

Results

Effects of FK-506 and rapamycin on blood urea nitrogen, serum creatinine and kidney microsomal P450 content

In the control rats (group A), mean blood urea nitrogen and serum creatinine concentrations on day 0 were 15·3 and 0·56 mg dL^{-1}, respectively. In group B (olive oil alone), on the same days, the mean blood urea nitrogen concentrations were 18·5 and 23·9 mg dL^{-1}, and the mean serum creatinine concentrations were 0·55 and 0·7 mg dL^{-1}, respectively (Table 1). There was no difference between the P450 content of kidney microsomes from groups A and B (Table 2). After treatment with cyclosporin (30 mg kg^{-1}/day; group C) mean blood urea nitrogen concentration rose to 31·0 and 78·8 mg dL^{-1} on days 7 and 14, respectively, and the mean serum creatinine concentration rose to 0·65 and 3·2 mg dL^{-1}, again respectively, indicating the development of nephrotoxicity (Table 1). Cyclosporin increased the specific P450 content of kidney cortex microsomes twofold (approx.; 135·6 ± 9·4 pmol mg^{-1}, Table 2) compared with groups A and B. FK-506 and rapamycin did not increase the mean serum concentrations of blood urea nitrogen and serum creatinine (Table 1). After treatment with FK-506 at 1 or 3 mg kg^{-1}/day (groups D and E) for 14 days, mean blood urea nitrogen concentrations were 27·5 and 30·4 mg dL^{-1}, respectively, and mean serum creatinine concentrations were 0·42 and 0·47 mg dL^{-1}, respectively (Table 1). After treatment with vehicle

Table 1. Effect of FK-506 and rapamycin on blood urea nitrogen and serum creatinine levels.

Group	Day 0		Day 7		Day 14	
	Blood urea nitrogen	Serum creatinine	Blood urea nitrogen	Serum creatinine	Blood urea nitrogen	Serum creatinine
A Untreated	15·3 ± 1·1	0·56 ± 0·07	15·7 ± 1·2	0·57 ± 0·07	16·3 ± 1·0	0·57 ± 0·07
B Olive oil alone	15·5 ± 1·2	0·55 ± 0·08	18·5 ± 1·3	0·55 ± 0·07	23·9 ± 1·9	0·70 ± 0·09
C Cyclosporin treatment (30 mg kg^{-1}/day)	15·3 ± 1·4	0·52 ± 0·09	31·0 ± 1·9	0·65 ± 0·08	78·8 ± 4·9	3·2 ± 0·11
D FK-506 treatment (1 mg kg^{-1}/day)	15·7 ± 1·6	0·57 ± 0·03	19·2 ± 1·9	0·55 ± 0·08	27·5 ± 3·0	0·42 ± 0·13
E FK-506 treatment (3 mg kg^{-1}/day)	18·0 ± 1·7	0·56 ± 0·07	20·5 ± 1·5	0·53 ± 0·06	30·4 ± 4·7	0·47 ± 0·06
F Vehicle alone	16·3 ± 2·2	0·58 ± 0·09	20·4 ± 2·4	0·62 ± 0·12	23·9 ± 1·7	0·60 ± 0·04
G Rapamycin treatment (0·3 mg kg^{-1}/day)	17·1 ± 1·1	0·63 ± 0·12	22·9 ± 3·8	0·54 ± 0·06	18·5 ± 2·0	0·55 ± 0·07
H Rapamycin treatment (3 mg kg^{-1}/day)	20·3 ± 2·4	0·60 ± 0·10	23·4 ± 2·3	0·66 ± 0·07	19·6 ± 1·9	0·58 ± 0·09

Each value (mg dL^{-1}) is the mean ± s.d. of results from five rats.

Table 2. Effect of FK-506 and rapamycin on the specific P450 content of kidney cortex microsomes of rats.

Group	Day 0	Day 7	Day 14
A Untreated	68·8 ± 5·0	69·3 ± 3·6	69·1 ± 3·5
B Olive oil alone	69·0 ± 3·7	67·7 ± 5·6	66·9 ± 3·9
C Cyclosporin treatment (30 mg kg^{-1}/day)	68·8 ± 5·9	111·3 ± 6·9*	135·6 ± 9·4*†
D FK-506 treatment (1 mg kg^{-1}/day)	64·7 ± 3·6	65·4 ± 6·9	66·2 ± 4·4
E FK-506 treatment (3 mg kg^{-1}/day)	63·2 ± 4·2	63·3 ± 7·4	66·7 ± 7·2
F Vehicle alone	64·8 ± 5·1	71·1 ± 5·7	78·8 ± 6·7
G Rapamycin treatment (0·3 mg kg^{-1}/day)	66·6 ± 4·2	70·3 ± 4·3	74·7 ± 7·2
H Rapamycin treatment (3 mg kg^{-1}/day)	62·9 ± 4·9	65·7 ± 3·7	66·9 ± 4·9

Each value (pmol (mg protein)$^{-1}$) is the mean ± s.d. of results from five rats. *$P < 0·05$ compared with olive oil alone. †$P < 0·05$ compared with untreated.

alone (Group F) or rapamycin at 0·3 or 3 mg kg^{-1}/day (groups G and H) for 14 days, mean blood urea nitrogen concentrations were 23·9, 18·5 and 19·6 mg dL^{-1} and mean serum creatinine concentrations were 0·60, 0·55 and 0·58 mg dL^{-1}, respectively (Table 1). In addition, the specific P450 content of kidney cortex microsomes in FK-506-treated rats (group D 66·2 ± 4·4 pmol mg^{-1}, group E 66·7 ± 7·2 pmol mg^{-1}) were not significantly different from those of the control group (69·1 ± 3·5 pmol mg^{-1}, Table 2). The specific P450 content of rats treated with vehicle alone (group F 78·8 ± 6·7 pmol mg^{-1}) or with rapamycin (group G 74·7 ± 7·2, group H 66·9 ± 4·9 pmol mg^{-1}) was no different from that of the untreated control group (69·1 ± 3·5 pmol mg^{-1}; Table 2).

Effects of FK-506 and rapamycin treatment on rat kidney cytochrome P450-linked monooxygenase activity

The laurate ω- and (ω-1)-hydroxylase activity of microsomes from kidney cortex isolated on day 14 from cyclosporin-treated rats was the same as that from rats given olive oil alone. On day 14 the activity of these enzymes in microsomes from rats treated with FK-506 was 68% (approx.) that of control levels in group D ($P < 0·05$) and 70% that of control levels in group E ($P < 0·05$) (Table 3). The enzyme activity in rats treated with vehicle alone (0·6 ± 0·11 nmol (mg protein)$^{-1}$) was reduced to 48% that of control levels (1·25 ± 0·1 nmol (mg protein)$^{-1}$); in rats treated with rapamycin enzyme activity was reduced to 67% of the control level in group G ($P < 0·01$) and to 72% of the control level in group H ($P < 0·01$). No dose-dependent differences were observed between groups D and E or between groups G and H (Table 3). PGA_1 ω-hydroxylase activity in rats treated with cyclosporin (group C) was reduced to 60% that in group B (olive oil alone). Activity in rats treated with FK-506 was increased twofold (approx.) in group D ($P < 0·05$) and 1·9-fold in group E ($P < 0·05$). The activity in rats treated with vehicle alone (group F) was increased to 1·7-fold (approx.) that of untreated controls (group A) whereas activity in rats treated with rapamycin was increased 1·9-fold (approx.) in groups G ($P < 0·05$) and H ($P < 0·05$) compared with untreated controls (group A), although differences between these groups and group F were not statistically significant. There were no differences between PGA_1 ω-hydroxylase activity in groups D and E (Table 3). Microsomal aminopyrine *N*-demethylase activity was increased approximately 2·5-fold in group C, 1·5-fold in group D ($P < 0·05$), 2·4-fold in group E ($P < 0·05$), 4·4-fold in group F ($P < 0·05$), 4·3-fold in group G ($P < 0·05$) and 3·9-fold in group H ($P < 0·05$) (Table 3). There was a significant dose-dependent difference between groups D and E, but not between groups G and H.

Western-blot analysis of rat kidney microsomes with antibodies against CYP2B4

No band was detected after analysis of kidney cortex microsomes from rats treated with olive oil alone, those treated with FK-506 (group E) or those receiving rapamycin (group H; Figure 1) whereas

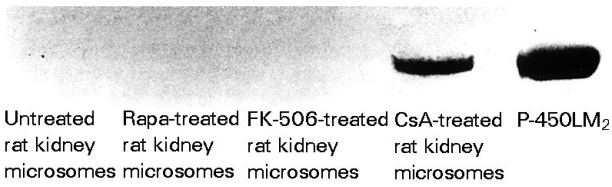

Figure 1. Western-blot analysis of rat kidney microsomes with antibody against CYP2B4. Rat kidney cortex microsomes (200 μg protein/well) and CYP2B4 (2 pmol) were subjected to electrophoresis. Lane 1, untreated; Lane 2, rapamycin-treated; Lane 3, FK-506-treated; Lane 4, cyclosporin-treated; Lane 5, CYP2B4.

Table 3. Effect of FK-506 and rapamycin treatment on laurate ω- and (ω-1)-hydroxylase, prostaglandin PGA1 ω-hydroxylase and aminopyrine *N*-demethylase activity in rat kidney cortex on day 14.

Group	Laurate ω- and (ω-1)-hydroxylase	Prostaglandin A_1 ω-hydroxylase	Aminopyrine *N*-demethylase
A Untreated	1·25 ± 0·10	0·037 ± 0·008	0·025 ± 0·013
B Olive oil alone	1·27 ± 0·10	0·038 ± 0·009	0·026 ± 0·014
C Cyclosporin treatment (30 mg kg^{-1}/day)	1·18 ± 0·14	0·024 ± 0·013*	0·063 ± 0·016*
D FK-506 treatment (1 mg kg^{-1}/day)	0·85 ± 0·17*	0·076 ± 0·011*	0·040 ± 0·015*
E FK-506 treatment (3 mg kg^{-1}/day)	0·88 ± 0·15*	0·073 ± 0·009*	0·060 ± 0·020*
F Vehicle alone	0·60 ± 0·11	0·064 ± 0·012	0·111 ± 0·016
G Rapamycin treatment (0·3 mg kg^{-1}/day)	0·84 ± 0·11†	0·072 ± 0·011	0·107 ± 0·014
H Rapamycin treatment (3 mg kg^{-1}/day)	0·90 ± 0·09†	0·073 ± 0·012	0·098 ± 0·009

The activity of the three monooxygenase enzymes is expressed as nmol products min^{-1} (mg microsomal protein)$^{-1}$. Each value is the mean ± s.d. of results from five rats. *$P < 0·05$ compared with olive oil alone. †$P < 0·05$ compared with vehicle alone.

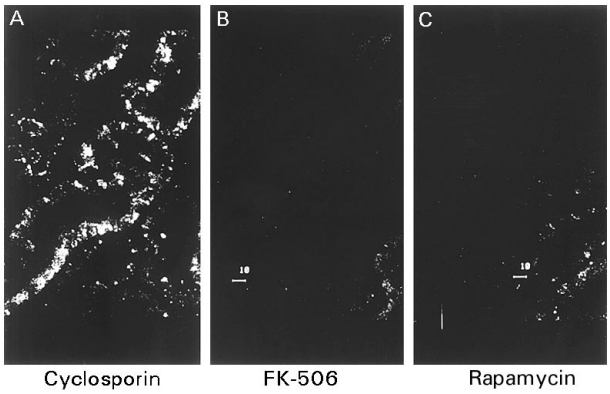

Figure 2. Histochemical study by immunofluorescence using antibody against CYP4A2 incubated with fluorescent isothiocyanate-labelled goat anti-guinea-pig IgG (dilution 1 : 500) overnight at 4°C.

analysis of kidney cortex microsomes from rats treated with cyclosporin (group C, Figure 1) resulted in a strong band, suggesting that a protein (P450) immunochemically related to CYP2B4 is inducible by cyclosporin, but not by FK-506 and rapamycin.

Histochemical study by immunofluorescence using antibodies against CYP4A2

Histochemical studies clearly revealed a form of P450 immunochemically related to CYP4A2 in the proximal tubules of rats treated with cyclosporin, indicating significant nephrotoxicity (Figure 2A). CYP4A2 was not detected in the kidneys of rats treated with FK506 or rapamycin (Figures 2B, C).

Discussion

FK-506 and rapamycin, the modes of action of which are very similar to that of cyclosporin, are effective and well-tolerated immunosuppressants in solid organ transplantation. However, these drugs are nephrotoxic and can induce dose-dependent deterioration of renal function, which can be reversible during the early post-transplantation phase. Although the pattern of nephrotoxicity of these drugs is essentially indistinguishable, both clinically and pathologically (McCauley 1993), it is generally considered that the immunosuppressive activity of FK-506 and rapamycin is much higher than that of cyclosporin, but that their nephrotoxicity is lower (Kino et al 1987; Ochiai et al 1987; Todo et al 1987). Changing from cyclosporin to FK-506 or rapamycin in kidney-transplanted patients with long-term cyclosporin-induced nephrotoxicity ameliorates graft dysfunction in the majority of patients (Hernandez-Herrera 1998). Some investigators have reported that changing from cyclosporin to FK-506 can reverse the deterioration of renal function which is secondary to acute cyclosporin nephrotoxicity (Hariharan et al 1996; Morrissey et al 1997; Pratschke et al 1997; Hernandez-Herrera 1998; Morris-Stiff et al 1998), although the mechanism of development of nephrotoxicity is unclear. To address this problem, we have studied the relationship between these drugs and renal P450 systems.

Recent studies have shown that cyclosporin is metabolized into various products by liver P450 systems. Some investigators (Imai et al 1980; Bertault-Peres et al 1987; Pichard et al 1991) showed that rabbit liver P450 (CYP3A6) catalyses the monohydroxylation, dihydroxylation and demethylation of cyclosporin at different sites. It is generally believed that cyclosporin is metabolized by CYP3A4 in the liver in man (Fromm et al 1996; Gan et al 1996; Pichard et al 1996; Watkins 1996; Zhou et al 1996). In rabbit and rat livers CYP2B4 and CYP4A2, respectively, metabolize many different drugs, in a manner similar to CYP3A in the liver in man.

Although several liver microsomal P450 enzymes have been isolated and characterized (Combalbert et al 1989; Shaw et al 1989) fewer isoforms of kidney P450 have been isolated (Machkov'a et al 1990; Massicot et al 1994; Nakamura et al 1994). Three different forms of P450, denoted CYP4A7 (P450Ka), CYP2B4 (P450Kb) and CYP1A1 (P448K), have been isolated from rabbit kidney cortex microsomes and characterized (Ogita et al 1982; Kusunose et al 1984). Of these, CYP2B4 (P450Kb) is activated by a variety of drugs, including aminopyrine, and is similar to CYP2B4 (P450LM2) of rabbit liver in its immunochemical characteristics. Little is known about the presence of forms of P450 immunochemically related to CYP2B4 (P450LM2) in rat kidney cortex microsomes. Recently, a form of P450, denoted CYP4A5 (P450Kd), has been isolated in a highly pure form from the kidney cortex microsomes of DEHP-treated rabbits (Kusunose et al 1989).

Previous studies have demonstrated that treatment of rats with cyclosporin induces at least one form of kidney P450, possibly related to rabbit liver CYP2B4. Treatment of rabbits with cyclosporin results in the induction of at least two different forms of P450 in the kidney, one of which is related to CYP2B4, and the other a fatty acid ω-hydroxylating P450 enzyme similar to CYP4A5. In this study, treatment of rats with cyclosporin for 14 days increased the total and specific content of P450 4·7-fold and twofold, respectively, in rat

kidney cortex microsomes. The same treatment resulted in a 2·5-fold increase in microsomal aminopyrine *N*-demethylase activity in the kidney. Hence, cyclosporin-inducible P450 might be associated with the monooxygenase activity of drugs such as aminopyrine. These results are consistent with the discovery of a significant increase in cytochrome P450 concentration in the kidneys of rats treated with cyclosporin (Mayer et al 1989). It is speculated that cyclosporin might contribute to the increase in cytochrome P450 concentration. Our data also supported this idea with regard to kidney P450, because treatment with cyclosporin resulted in a 2·5-fold increase in microsomal aminopyrine *N*-demethylase activity in the kidney. In the current study, we examined the effects of three different immunosuppressive drugs on renal P450 enzymes. Clinically effective dosages of these drugs are less than $10\,mg\,kg^{-1}$/day of cyclosporin, less than $0{\cdot}1\,mg\,kg^{-1}$/day of FK-506, and $0{\cdot}3\,mg\,kg^{-1}$/day of rapamycin. We chose dosages that are effective and not nephrotoxic, ranging from 3 to 10 times the clinical dosages (cyclosporin $30\,mg\,kg^{-1}$/day, FK-506 $3\,mg\,kg^{-1}$/day, rapamycin $3\,mg\,kg^{-1}$/day; Morris et al 1990; Kahan et al 1991; Platz et al 1991; Stepkowski et al 1991; Andoh et al 1996). Nephrotoxicity occasionally occurs when a high dose of FK-506 or rapamycin is administered. In this study, $3\,mg\,kg^{-1}$ FK-506 or $3\,mg\,kg^{-1}$ rapamycin was used to induce nephrotoxicity. However, blood urea nitrogen and serum creatinine concentrations were not elevated on day 14 after treatment with FK-506 or rapamycin. Histological studies did not reveal clear changes in renal tubules or glomeruli of rats treated with FK-506 or rapamycin. These findings support the hypothesis that FK-506 and rapamycin lead to less incidence of nephrotoxicity than cyclosporin. Although the specific P450 content of kidney cortex microsomes from rats treated with FK-506 and rapamycin was not significantly elevated, aminopyrine *N*-demethylase activity was increased in FK-506-treated rats, and the difference between findings for different groups might reflect the dose of FK-506 administered. PGA_1 ω-hydroxylase activity increased in rats treated with FK-506, but decreased in those treated with cyclosporin. Rapamycin treatment had no effect. Laurate ω- and (ω-1)-hydroxylase activity increased in rats treated with rapamycin and decreased in those treated with FK-506. Although it has previously been reported that FK-506 and rapamycin are primarily metabolized by CYP3A4 in the liver of both man and rat (Sattler et al 1992), that study did not examine renal P450 systems in rats treated with FK-506 or rapamycin. In our study the treatment of rats with FK-506 or rapamycin did not increase the specific P450 content of the renal cortex, indicating that FK-506 and rapamycin might not be metabolized in the kidney. Furthermore, Western-blot analysis employing guinea-pig antibodies against CYP2B4 revealed that one form of the cyclosporin-inducible P450 in renal cortex is immunochemically related to CYP2B4 (P450LM2). Treatment of rats with FK-506 or rapamycin did not induce a form of kidney cortex microsomal P450 immunochemically related to CYP2B4. The histochemical study clearly revealed a type of P450 immunochemically related to CYP4A2 (P450K2) in the proximal tubules of rats. CYP4A2 (P450K2) has been separated in a highly pure form from the kidney cortex microsomes of normal rats (Yoshimoto et al 1986). Cyclosporin increases arachidonic acid ω-hydroxylation activity by induction of CYP4A2; this specific induction of CYP4A2 might be related to cyclosporin-induced nephrotoxicity and elevated blood pressure, because ω-hydroxyarachidonic acid is a potent vasoconstrictor (Nakamura et al 1994). These two forms of P450 metabolize the ω- and (ω-1)-hydroxylation fatty acids and have no activity with xenobiotics such as aminopyrine.

Although neither FK-506 nor rapamycin was metabolized in renal cytochrome P450 systems, it remains unknown why these agents have different effects on cytochrome P450-linked monooxygenase activity. It has been reported that both rapamycin and FK-506 bind to a family of cytoplasmic and membrane-bound proteins called FK-binding proteins (FK BPs) which are distinct from the cyclophilins and do not bind cyclosporin (Siekierka et al 1989). The molecular weights, localization and activity of these FK binding proteins have been characterized and molecular studies have shown that it is largely FK BP12, a 12-kDa cytosolic protein with peptidyl-prolyl *cis-trans* isomerase (PPIase) activity, that is involved in T cell activation and mediates the immunosuppressive effects of FK-506 (Bram et al 1993). FK BP13 is the major rapamycin-binding protein. The structural characteristics of these two FK binding protein molecules might lead to different effects of these agents on cytochrome P450-linked monooxygenase activity. Although, unfortunately there is no direct evidence that cyclosporin-mediated increases in renal cytochrome P450 lead to nephrotoxicity, this possibility is consistent with the finding that cytochrome P450 in the kidney is associated with biotransformation of endogenous compounds such as prostaglandins rather than metabolism of this drug (Okita et al 1981); alterations in prostaglandin biotransformation might be intimately linked to the nephrotoxicity of cyclosporin.

In conclusion, this study has shown that although cyclosporin has a strong effect on the renal P450 systems and induces a form of kidney cortex microsomal P450, FK-506 and rapamycin have no remarkable effect on the induction of renal P450. The extent of P450 induction by cyclosporin in the kidney correlates with the extent of renal impairment. FK-506 and rapamycin might induce significantly less renal P450, indicating lower incidence of nephrotoxicity.

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