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INDUCTION OF CYTOCHROME P450 ISOZYMES IN RAT RENAL MICROSOMES BY CYCLOSPORIN A

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Abstract—To examine the effects of cyclosporin A (CsA) on renal cytochrome P450 forms, CsA was administered to rats, and the renal levels of P450 were determined by immunoblotting. CsA treatment for 17 days increased total renal P450 content by 40% with a concomitant elevation of the ω- and (ω-1)-hydroxylation activities of lauric acid. Arachidonic acid ω-hydroxylation activity was also induced 2-fold by treatment with CsA for 17 days. Among the P450 forms, CYP4A2 was induced significantly, whereas CYP2C23, CYP4A1 and CYP4A8 were unaffected. These changes were accompanied by slight but significant increases in blood urea nitrogen and systolic blood pressure. These data suggest that CsA increased arachidonic acid ω-hydroxylation activity by the induction of CYP4A2. The specific induction of CYP4A2 may be related to CsA-induced nephrotoxicity and elevated blood pressure, because ω-hydroxyarachidonic acid is a potent vasoconstrictor.

Key words: renal P450; cyclosporin A; arachidonic acid metabolism; blood pressure

CsA§ is an immunosuppressive drug that is widely used in organ transplantation. This drug has adverse side-effects in both experimental animals and humans [1–4], and its nephrotoxicity is often the dose-limiting factor in its use [4]. Although the mechanism of nephrotoxicity remains to be elucidated, it is speculated that renal vasoconstriction is responsible for the CsA-induced nephrotoxicity because its nephrotoxic effects can be diminished by coadministration of a vasodilator [5].

Administration of CsA to rats induces total cytochrome P450 in renal microsomes, and the contribution of P450 to the nephrotoxicity caused by CsA has been suggested [6]. Renal P450 plays a role in the biotransformation of biologically active substances such as leukotrienes, prostaglandins, and fatty acids such as arachidonic acid and lauric acid [7–10]. Renal P450s can produce ω-hydroxyarachidonic acid, (ω-1)-hydroxyarachidonic acid, and epoxyeicosatrienoic acids (EETs) from arachidonic acid. ω-Hydroxyarachidonic acid is a potent vasoconstrictor, and 4,5-EET is a stimulator of Na^+/K^+-ATPase [11, 12]. These findings have led to the previous suggestions that induction of renal P450 alters the metabolism of arachidonic acid and that renal P450s may contribute to the regulation of renal function [6]. We purified some forms of P450 from rat renal microsomes and investigated hormonal regulation of these forms and changes in the levels of these forms in pathophysiological conditions, such as hypertension [13, 14], which suggest that CYP4A2 may contribute to hypertension.

In this study, we determined which forms of P450 are induced in the rat kidney by CsA and elucidated their possible roles in CsA-induced renal dysfunction.

MATERIALS AND METHODS

Chemicals. CsA was supplied by the Sandoz Co. (Basel, Switzerland). Sodium laurate and ω-hydroxylauric acid were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). (ω-1)-Hydroxylauric acid was synthesized as described previously [15]. [^{14}C]Arachidonic acid was obtained from the Amersham Corp. (Buckinghamshire, U.K.). Other reagents and organic solvents were obtained from Wako Pure Chemicals (Tokyo, Japan).

Preparation of microsomes and immunochemical methods. Male Sprague–Dawley rats, weighing 230–250 g, from Clea Japan Inc. (Tokyo) were given CsA (50 mg/kg/day) dissolved in olive oil p.o. once daily for 4, 10, and 17 days. Control rats received equal volumes of olive oil. Renal microsomes were prepared by differential centrifugation as described previously [16]. P450 K-2, K-4, and K-5 were purified from renal microsomes of untreated male rats as reported previously [17]. Antibodies against P450 K-2, K-4, and K-5 were raised as described previously in a female Japanese white rabbit obtained from Biotech (Saga, Japan) and were characterized previously [17]. Based on the results of N-terminal amino acid sequence analysis and immunochemical study, P450 K-2, K-4, and K-5 correspond to CYP2C23, 4A8, and 4A2, respectively [17–20]. CYP4A1 was purified from hepatic microsomes of rats treated with clofibrate by a method similar to

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§ Abbreviations: CsA, cyclosporin A; and BUN, blood urea nitrogen.

Table 1. Effects of CsA on BUN and blood pressure of rats

		N	BUN (mg/dL)	Blood pressure (mm Hg)
Control	(4 days)	5	16.7 ± 2.2	118 ± 12.7
CsA	(4 days)	6	17.1 ± 1.7	111 ± 9.9
Control	(10 days)	5	14.9 ± 1.1	117 ± 8.1
CsA	(10 days)	6	18.2 ± 2.1*	129 ± 3.9†
Control	(17 days)	5	15.2 ± 0.6	116 ± 6.0
CsA	(17 days)	6	26.2 ± 3.7†	129 ± 10.2*

BUN was measured in duplicate samples from 5–6 different animals. Cystolic blood pressure was measured five times. Values are expressed as means ± SD. Abbreviations: BUN, blood in urea nitrogen; and CsA, cyclosporin A.

* Significantly different from control, $P < 0.05$.

† Significantly different from control, $P < 0.01$.

that used to purify CYP3A2 [21]. Anti-CYP4A1 antibody was prepared as described above. Anti-CYP4A1 antibody weakly cross-reacted with CYP4A2 and 4A3 and was purified by affinity chromatography with CYP4A2- and 4A3-binding Sepharose 4B, as previously reported [22]. Purified CYP4A1 antibody reacted only with CYP4A1. Individual cytochrome P450 isozymes were assayed immunochemically as described in Refs. 17 and 22. After SDS–PAGE with a 7.5% polyacrylamide gel, proteins were transferred from the gel to a nitrocellulose sheet (Bio-Rad Laboratories, Richmond, CA, U.S.A.) in a buffer containing 100 mM Tris, 192 mM glycine and 20% methanol. The nitrocellulose membrane was treated with antibody and stained with the use of a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, U.S.A.). Stained bands were quantified by densitometry as a standard with purified P450s.

Other methods. The activities of lauric acid and arachidonic acid hydroxylation were measured as described previously [15, 23]. Systolic blood pressure was measured by the tail cuff method. BUN was measured using a BUN assay kit (Wako). Cytochrome P450 was assayed spectrometrically [24]. Protein concentration was measured by the method of Lowry *et al.* [25].

RESULTS

Changes in BUN and blood pressure resulting from treatment with CsA. Table 1 summarizes the BUN and blood pressure values of rats treated with CsA for 4, 10, or 17 days. BUN increased significantly after CsA treatment for 10 and 17 days, suggesting that kidney function was declining. The blood pressure of the rats was also increased significantly by CsA treatment for 10 or 17 days. Effects of CsA on body and kidney weights appeared after 4 days of treatment (data not shown), whereas both BUN and the blood pressure of rats treated for 4 days were not significantly different from those of control rats.

Effects of CsA treatment on lauric acid and arachidonic acid hydroxylation activities. The total content of P450 measured photometrically in renal microsomes was increased by CsA treatment for 17 days (Table 2). Lauric acid ω-hydroxylation is catalyzed mainly by CYP4A forms in the rat kidney [17, 26]. Lauric acid ω-hydroxylation activity was increased 38% by long-term treatment with CsA. Lauric acid (ω-1)-hydroxylation is catalyzed by CYP4A forms and by CYP2C23 [17, 23]. Treatment for 10 or 17 days also induced (ω-1)-hydroxylation activity. Arachidonic acid ω- and (ω-1)-hydroxylation activities were induced by treatment for 10 or 17 days. Note that CsA treatment for 17 days induced arachidonic acid ω-hydroxylation activity 2-fold.

Changes in the levels of P450 forms produced by treatment with CsA. The levels of four forms of P450 (CYP2C23, 4A1, 4A2, and 4A8) in the renal microsomes of rats treated with CsA were measured (Fig. 1 and Table 3). CYP2C23 is a minor P450 in rat renal microsomes and catalyzes the epoxidation of arachidonic acid [18]. This form was not induced by CsA. CYP4A2 is a major P450 in rat renal microsomes and catalyzes the ω-hydroxylation of arachidonic acid [23]. This P450 was induced by treatment with CsA for 10 or 17 days, but CYP4A1 and 4A8 were not, even though these three forms

Table 2. Changes in the levels of renal P450s by treatment with CsA

		N	Total P450 (nmol/mg)	Lauric acid (nmol/min/mg)		Arachidonic acid (nmol/min/mg)	
				ω-1	ω	ω-1	ω
Control	(4 days)	5	0.069 ± 0.007	0.24 ± 0.092	0.70 ± 0.166	0.105 ± 0.019	0.173 ± 0.031
CsA	(4 days)	6	0.081 ± 0.013	0.29 ± 0.024	0.76 ± 0.135	0.096 ± 0.018	0.171 ± 0.081
Control	(10 days)	5	0.066 ± 0.009	0.22 ± 0.026	0.68 ± 0.075	0.099 ± 0.026	0.170 ± 0.018
CsA	(10 days)	6	0.082 ± 0.015	0.34 ± 0.042*	0.80 ± 0.042*	0.138 ± 0.008†	0.248 ± 0.026*
Control	(17 days)	5	0.071 ± 0.010	0.28 ± 0.036	0.79 ± 0.078	0.117 ± 0.014	0.176 ± 0.019
CsA	(17 days)	6	0.096 ± 0.005*	0.42 ± 0.078*	1.09 ± 0.085*	0.162 ± 0.020*	0.329 ± 0.023*

Each value is the mean ± SD from 5–6 animals. Key: ω and ω-1 indicate the hydroxylation sites of lauric acid and arachidonic acid; CsA, cyclosporin A.

† Significantly different from control, $P < 0.05$.

* Significantly different from control, $P < 0.01$.

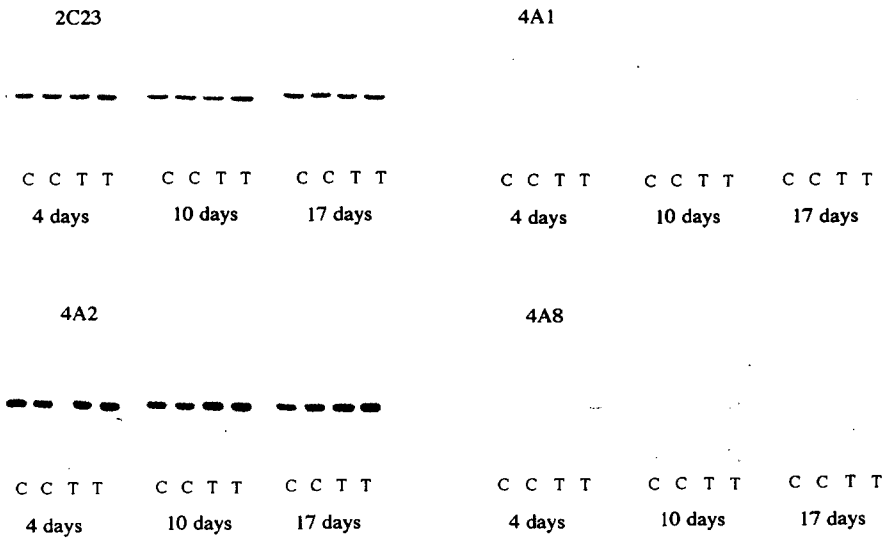

Fig. 1. Immunoblots of renal microsomes of rats treated with CsA. Renal microsomes (20 μg for CYP2C23, 40 μg for CYP4A1 and 4A8, and 5 μg for CYP4A2 antibody) from control rats and from those treated with CsA for 4, 10, or 17 days were resolved by SDS–PAGE. Membranes were stained immunochemically with the antibodies against P450s indicated on the top of the membranes. Key: C, renal microsomes of control rats corresponding to each treatment; T, renal microsomes of rats treated with CsA.

Table 3. Levels of CYP2C23, 4A1, 4A2 and 4A8 in rat kidney microsomes

			P450 forms (pmol/mg)			
		N	CYP2C23	CYP4A1	CYP4A2	CYP4A8
Control	(4 days)	5	12.1 ± 2.9	0.3 ± 0.08	65.1 ± 10.2	2.8 ± 1.0
CsA	(4 days)	6	11.5 ± 3.0	0.3 ± 0.10	78.4 ± 12.2	2.4 ± 0.7
Control	(10 days)	5	14.0 ± 1.2	0.3 ± 0.05	65.5 ± 8.0	3.1 ± 1.0
CsA	(10 days)	6	13.0 ± 2.1	0.4 ± 0.09	88.3 ± 5.2*	3.7 ± 1.8
Control	(17 days)	5	11.3 ± 2.6	0.4 ± 0.09	78.6 ± 3.3	3.1 ± 0.9
CsA	(17 days)	6	10.5 ± 2.9	0.5 ± 0.08	98.0 ± 4.2*	3.0 ± 0.7

Each value is the mean ± SD from 5–6 animals. CsA, cyclosporin A.
* Significantly different from control, $P < 0.01$.

belong to the same gene family. The increase in the level of CYP4A2 after 10 days of CsA treatment paralleled the effect of CsA treatment on BUN and blood pressure. The increase in arachidonic acid ω-hydroxylation activity reflects the increased levels of CYP4A2.

DISCUSSION

In this study, we investigated the effects of CsA on renal P450 and found that it specifically induced CYP4A2, a major renal form, whereas CYP2C23, CYP4A1 and CYP4A8 were unaffected. Thus, the increase in CYP4A2 is solely responsible for that of the total content of renal P450 induced by CsA. CsA causes a decrease in renal blood flow by affecting the metabolism of vasoactive substances in the kidney [27]. CYP4A2 can produce ω-hydroxyarachidonic acid, a potent vasoconstrictor, from arachidonic acid [12, 23]. Induction of arachidonic acid ω-hydroxylation activity by CsA was accompanied by a slight but significant increase in blood urea nitrogen and elevated blood pressure. The increase in arachidonic acid ω-hydroxylation activity reflects the increased levels of CYP4A2. Therefore, the increased level of CYP4A2 may be involved in the CsA-induced nephrotoxicity via the enhanced production of ω-hydroxyarachidonic acid. The possible role of ω-hydroxyarachidonic acid has been assessed in spontaneously hypertensive rats (SHR), which have

high levels of CYP4A2, by comparing them with normotensive rats (WKY) [14]. It was found that a decrease in the renal P450 level prevents the increase in blood pressure of SHR [28]. In this study, CsA caused a slight but significant elevation of blood pressure that may also be related to the increased CYP4A2.

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