Increased Excretion of Urinary 20-HETE in Rats With Cyclosporine-Induced Nephrotoxicity

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Abstract. The present study examined the contribution of 20-hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) in cyclosporine A (CsA)-induced renal nephrotoxicity. Treatment of rats with CsA (50 mg/kg) for 9 days induced renal damage as indicated by marked increase in urine flow (from 9.0 ± 0.3 ml/day to 46.6 ± 7.1 ml/day) and a 3–5-fold rise in blood urea nitrogen (BUN) levels. The urinary excretion of 20-HETE increased from 164 ± 5 ng/day (N = 5) to 2432 ± 290 ng/day (N = 5, P < 0.01) after 9 days of CsA treatment. The increase in the urinary excretion of 20-HETE in the CsA treated rats was highly correlated with the increase in BUN levels (r = 0.819, P < 0.001) and urine volume (r = 0.832, P < 0.001). Immunohistochemical examination of kidney revealed that expression of cytochrome P450 4A (CYP4A) protein was markedly enhanced in the proximal tubules of CsA-treated rats. These results indicate that CsA-induced nephrotoxicity in rats is associated with a marked elevation in the renal production of 20-HETE and that 20-HETE may contribute to the pathophysiological condition of CsA-induced nephrotoxicity.

Keywords: cyclosporine A, renal injury, nephrotoxicity, 20-HETE, cytochrome P450 4A

Introduction

Cyclosporine A (CsA) is a prototypical nephrotoxin (1, 2) that is still widely used in the treatment of autoimmune diseases and for immuno-suppression following organ transplantation.

Coffman et al. (3) reported that CsA-induced nephrotoxicity was associated with an increase in the renal production of prostaglandins and thromboxane. CsA also stimulates the release of endothelin-1 (ET-1) from endothelial, tubular, and mesangial cells (4, 5) in rats and has been associated with increased expression of angiotensin II (ANG II) type I receptors in the kidney. Activation of each of these pathways could promote an increase in renal vascular tone. The observations that an ET-1-receptor antagonist and inhibitors of the renin angiotensin system attenuate the decline in renal function following administration of CsA provide further support for a role for endothelin and ANG II in cyclosporine induced nephrotoxicity (6 – 9).

Several investigators have reported that cytochrome P450 4A (CYP4A) isoforms that catalyze the formation of 20-hydroxy 5,8,11,14-eicosatetraenoic acid (20-HETE) are highly expressed in the kidney (10, 11) and that 20-HETE is a primary metabolite of arachidonic acid (AA) produced in the kidney of rats (12, 13). 20-HETE inhibits sodium transport in isolated perfused rabbit proximal tubules (14) and plays a major role in the regulation of chloride transport in the thick ascending limb of Henle (TALH) (15, 16). 20-HETE is also a potent constrictor of renal arterioles in rats (17, 18) and dogs (19) and the vasoconstrictor response to 20-HETE is mediated by the blockade of large conductance Ca²⁺-activated K⁺-channels (19, 20). Recent studies have also indicated that the production of 20-HETE is stimulated by ET-1 (21, 22) and ANG II (23 – 25) and that the elevation in the levels of 20-HETE in vascular smooth muscle cells potentiates the response to these vaso-
constrictors in the renal vasculature. There is one report that CsA increases the expression of CYP4A enzymes and the omega-hydroxylation of fatty acids in the kidney of rats (26). However, little is known about the role of 20-HETE in the pathogenesis of CsA-induced nephrotoxicity. Thus, the present study examined the time course of changes in the urinary excretion of 20-HETE and the renal expression of CYP4A protein during the development of CsA-induced nephrotoxicity in rats.

**Materials and Methods**

**CsA-induced nephropathy model in rats**

Experiments were performed in Male Sprague-Dawley rats (8-week-old; Japan SLC, Inc., Shizuoka). The rats were given daily intraperitoneal injections of CsA dissolved in corn oil (50 mg/kg per day) for 2, 6, and 9 days according to the method of Tariq et al. (27). Control rats received injections of an equal volume of (corn oil solution). Blood samples (0.3 ml) were drawn from the jugular vein at before and after 2, 6, and 9 days of CsA treatment. These samples were used to measure serum creatinine and blood urea nitrogen (BUN) concentration using a Hitachi 7150 biochemical analyzer. Urine was collected before and after 2, 6, and 9 days of CsA treatment by placing the rats in metabolic cages for 24 h. Urine samples were collected into the bottle on dry ice. All studies were reviewed by the Taisho Pharmaceutical Co., Ltd. Animal Care Committee and meet the Japanese Experimental Animal Research Association standards, as defined in the Guidelines for Animal Experiments (1987).

**Measurement of urinary levels of 20-HETE by fluorescent HPLC assay**

Urinary 20-HETE levels were measured according to the method of Maier et al. (28). In brief, an internal standard, 20-hydroxyeicosa 6(Z),15(Z)-dienoic acid (WIT0002), was added to the urine sample and then acetonitrile and acetic acid were added to make a final concentration of 20% and 0.05%, respectively. The urine sample was loaded onto a Sep-Pak column (Waters, Milford, MA, USA) and lipids were eluted with 100% acetonitrile. Acetonitrile was evaporated and the lipids were redissolved in 82% methanol, 18% H$_2$O, and 0.1% acetic acid. 20-HETE and WIT0002 were separated by reversed-phase HPLC (Symmetry C18 column, Waters) connected with a fluorometric detector (RF-10A; Shimadzu, Kyoto), with methanol/water/acetic acid (82:18:0.1 [vol/vol/vol]) at a rate of 1 ml/min at 40°C. The amount of 20-HETE in the sample was determined by comparing the area of the 20-HETE peak to that of the internal standard.

**Statistical analyses**

Means ± S.E.M. are presented. The significance of differences between the vehicle- and CsA-treated rats at corresponding time points was evaluated using a the paired t-test. A P value <0.05 was considered to be statistically significant. The statistical analyses were performed by using Origin 6.1J (OriginLab Corp., Northampton, MA, USA).
Drugs

Cyclosporine was purchased from Novartis Pharma K.K. (Tokyo). 20-HETE was obtained from Sigma Chemical Co. KIT002 was synthesized by the Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd. (Saitama).

Results

The effect of CsA treatment on rat serum urea, creatinine, and urinary parameters

The effects of CsA on BUN, serum creatinine concentrations, and urine volume are presented in Fig. 1. BUN levels increased significantly after 2, 6, and 9 days of treatment, with CsA Serum creatinine levels and urine volume also increased significantly in CsA-treated rats (Fig. 1). Urinary osmolality in vehicle- and CsA-treated rats at Day 9 were 1068.8 ± 64.7 mosmol/kgH2O (N = 8) and 385.3 ± 77.4 mosmol/kgH2O (N = 8, P < 0.01). Urinary protein excretion in vehicle- and CsA-treated rats at Day 9 were 7.0 ± 0.4 mg/day per 100 g body weight (N = 8) and 1.9 ± 0.3 mg/day per 100 g body weight (N = 7, P < 0.01). Mean systolic blood pressure of vehicle-treated and cyclosporine-treated rats at Day 9 were 116.9 ± 1.3 mmHg (N = 9) and 126.4 ± 2.9 mmHg (N = 9, P < 0.01).

The effect of CsA on the amount of urinary excretion of 20-HETE

The results of these experiments are presented in Fig. 2. The urinary excretion of 20-HETE progressively increased in rats treated with CsA. After 9 days of treatment, 20-HETE excretion was 15-fold higher than the control and that seen in vehicle-treated rats.

The relationship between 20-HETE excretion and the degree of renal damage as reflected by the rise in BUN levels and urine volume in CsA-treated rats is presented in Fig. 3. The urinary excretion of 20-HETE was highly correlated (r = 0.819, P < 0.001) with increase in BUN levels, an index of the glomerular filtration, and with urine volume, which reflects inhibition of tubular reabsorption (r = 0.832, P < 0.001). The urinary excretion of 20-HETE also strongly correlated with increase in serum creatinine concentration (r = 0.814, P < 0.001).

Effect of CsA on the renal expression of CYP4A protein

CsA treatment for 9 days produced a marked nephrotoxicity associated with dilation, atrophy of proximal tubules, the formation of tubular casts, sloughing of
tubular epithelial cells, and thickening of the tubular basement membranes. In the renal cortex of control rats, the expression of CYP4A was limited to the proximal tubules and TALH (Fig. 4A). There was very little expression of CYP4A protein in the outer and inner medulla. There was a marked increase in the staining of proximal tubules in rats treated with CsA for 9 days (Fig. 4B). CsA had no discernable effect on the expression of CYP4A protein in the outer or inner medulla (data not shown).

A comparison of the intensity of CYP4A protein in the control and CsA-treated rats is presented in Fig. 5. CsA treatment for 6 or 9 days markedly increased both the intensity and area of the expression of CYP4A staining in the renal cortex.

Discussion

20-HETE is the predominant natriuretic eicosanoid and a potent vasoconstrictor of the renal microvessels.
(17–19). Recent studies indicate that ANG II stimulates the formation of 20-HETE in rabbit isolated kidney (24) and in rat renal microvessels (25) and that 20-HETE contributes to both the acute and chronic pressor actions of ANG II (23). Endothelin-1 (ET-1) also stimulates the formation of 20-HETE and 20-HETE contributes to the renal vasoconstrictor response to this agonist as well (21, 22). Thus, the present study examined that the changes in urinary excretion of 20-HETE and immunohistochemistry of CYP4A protein in kidneys from rats with CsA-induced nephrotoxicity because elevations in renal ANG II (29–31) and ET-1 levels (24, 31) have been implicated in mediating the decline in renal function in this model.

The results indicate that the urinary excretion of 20-HETE is markedly elevated in rats treated with CsA for 6 or 9 days. This observation was confirmed by the finding that CsA treatment for 6 and 9 days markedly increased the expression of CYP4A protein in the proximal tubules. Interestingly the elevation in 20-HETE excretion was highly correlated with the increase in BUN levels (r = 0.819, P < 0.001), an index of the decline in glomerular function, and the degree of polyuria, reflected by the increase in urine volume (r = 0.832, P < 0.001) (Fig. 3). 20-HETE inhibits sodium transport in isolated perfused rabbit proximal tubules (14). 20-HETE also plays a major role in the regulation of chloride transport in the TALH (15, 16). Thus, the rise in 20-HETE, especially in proximal tubules, may also contribute at least partially to the progressive inhibition of tubular reabsorption and polyuria seen in CsA treated rats. 20-HETE is a potent renal vasoconstrictor that potentiates the vasoconstrictor actions of ET-1 and ANG II by depolarizing vascular smooth muscle through blockade of Ca2+-activated K+ channels. The strong correlation between the elevations in BUN and serum creatinine concentration and 20-HETE excretion suggests that elevations in renal levels of 20-HETE may contribute to the enhanced renal vascular tone associated with this model.

In a previous study, Nakamura et al. (26) reported that the expression of CYP4A2 protein and the production of omega-hydroxy arachidonic acid were induced in the kidney of rats treated with CsA. We confirmed using immunohistochemistry in the present study, that the expression of CYP4A protein is markedly elevated in the proximal tubule of rats treated with CsA. The mechanism by which expression of CYP 4A protein is increased in kidneys of CsA-treated rats is unknown. However, previous studies have reported that ANG II (29–31) and ET-1 (24, 31) levels are elevated in the kidney following administration of CsA and both of these factors are known to induce the expression of CYP4A protein and stimulate the formation of 20-HETE (21–23).

In conclusion, the present study indicates that the expression of CYP4A protein in the kidney and the urinary excretion of 20-HETE, a natriuretic and vasoconstrictor eicosanoid are elevated in rats with CsA-induced nephrotoxicity.

References
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