Inhibition of Calbindin D$_{28K}$ Expression by Cyclosporin A in Rat Kidney: The Possible Pathogenesis of Cyclosporin A-Induced Hypercalciuria

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Abstract. A recent study by Steiner et al. (Biochem Pharmacol 51: 253–258, 1996) demonstrated a decreased calbindin D$_{28K}$ expression in the kidneys of cyclosporin A (CsA)-treated rats. To evaluate the association of renal calcium handling with calbindin D$_{28K}$ expression in CsA-treated rats, two separate experiments (vehicle [VH] versus CsA groups, 1,25-dihydroxyvitamin D$_3$ [VitD] versus VitD + CsA groups) were done simultaneously. CsA (25 mg/kg per d, subcutaneously) and VitD (0.5 µg/kg per d, subcutaneously) were given for 7 d. The CsA group showed decreased serum calcium, increased urine calcium excretion, and decreased calbindin D$_{28K}$ protein level and immunoreactivity compared with the VH group. The VitD + CsA treatment decreased serum calcium, increased urine calcium excretion, and decreased calbindin D$_{28K}$ protein level and immunoreactivity compared with the VitD alone. CsA treatment did not affect the serum parathyroid hormone and VitD levels. This study demonstrates an association of calbindin D$_{28K}$ expression with the urinary calcium excretion in CsA-treated rats, and suggests that decreased calbindin D$_{28K}$ expression may play a role in renal calcium wasting.

Cyclosporin A (CsA) remains one of the most important immunosuppressive drugs in the management of organ transplantation and autoimmune disease. Despite its great benefits, a major side effect of CsA is nephrotoxicity (1,2). CsA causes renal tubular injury and tubular dysfunction. Of these, renal magnesium wasting is a well known complication (3–6), but little is known about the effect of CsA on renal calcium handling.

In general, most of the filtered calcium (approximately 60%) is reabsorbed in the proximal tubule and the remainder is reabsorbed in the medullary thick ascending limb of Henle’s loop (mTAL), the distal convoluted tubule, and the connecting segment. Calcium reabsorption in the proximal tubule and mTAL is largely passive and paracellular. In contrast, calcium reabsorption in the more distal segments is active and transcellular, and this is where the homeostatic control of calcium reabsorption occurs (7).

The calcium binding protein (calbindin) plays a significant role in the process of calcium transport (8–10). Two distinct subclasses of this protein with relative molecular masses of 9,000 and 28,000 have been described. The calbindin D$_{9K}$ is present in high concentrations in the proximal small intestine and facilitates intestinal calcium absorption (11). The calbindin D$_{28K}$ is expressed in the distal nephron segments (12,13) of the rat kidney and is assumed to take part in the process of calcium reabsorption (14,15).

1,25-dihydroxyvitamin D$_3$ (VitD) is an important inducer of calbindin D$_{28K}$ in the distal tubule (16–18). VitD, which regulates intestinal calcium absorption, bone calcium reabsorption, and renal calcium reabsorption, is activated in the renal proximal tubule by parathyroid hormone (PTH), and activated VitD induces the transcription of calbindin D$_{28K}$ in the distal tubule. This cascade process between VitD and calbindin D$_{28K}$ in the kidney strongly suggests a role for calbindin D$_{28K}$ in calcium absorption in distal tubule.

Recent studies by Steiner et al. (19,20) demonstrate a decreased calbindin D$_{28K}$ expression in the kidneys of long-term CsA-treated rats. However, the effects of CsA on segmental and cellular sites of calbindin D$_{28K}$ expression and renal calcium handling are unknown. In this study, we hypothesized that CsA might inhibit calbindin D$_{28K}$ expression in the distal nephron and that this might affect distal calcium reabsorption. To test this hypothesis, we examined the association of calbindin D$_{28K}$ expression and renal calcium handling in an acute model of CsA nephrotoxicity in rats treated with or without exogenous VitD, which is a well known inducer of calbindin D$_{28K}$ expression.

Materials and Methods

Animals

Adult male Sprague Dawley rats weighing 200 to 225 g were housed in individual cages in a temperature- and light-controlled
environment. All rats received a low-salt diet (0.05% sodium; Teklad, Madison, WI), which is known to accelerate the time course and magnify the structural and functional effects of CsA in rats (21).

Drugs
CsA, provided by Sandoz Research Institute (East Hanover, NJ), was diluted in olive oil to a final concentration of 25 mg/ml. VitD (Bonkey, Yu Han Pharmaceutical Co., Seoul, Korea) was dissolved in sterile water to a final concentration of 1 mg/ml.

Experimental Groups
Experiment 1 (vehicle versus CsA groups) and experiment 2 (VitD versus VitD + CsA groups) were done simultaneously. In the vehicle (VH) group, rats received a daily subcutaneous injection of olive oil, 1 mg/kg, for 7 d (n = 7). In the CsA group, rats received a daily subcutaneous injection of CsA, 25 mg/kg, for 7 d (n = 7). In the VitD group, rats received a daily subcutaneous injection of VitD, 0.5 μg/kg, for 7 d (n = 7). In the VitD + CsA group, rats received a daily subcutaneous injection of CsA, 25 mg/kg, and VitD, 0.5 μg/kg, for 7 d (n = 7).

Experimental Protocol
Weight-matched pairs of rats were randomly assigned to the different treatment groups. Daily body weight and food intake were recorded. Rats were fed the exact amount of food consumed the day before. At the end of each treatment period, 24-h quantitative urine samples were collected in metabolic cages (Nalge Co., Rochester, NY). The next day animals were anesthetized with ketamine, and a blood sample and tissue specimens were obtained. After opening the abdomen through a midline incision, the abdominal aorta was cannulated retrogradely below the renal arteries with an 18-gauge needle. With the aorta occluded by ligation above the renal arteries and renal veins opened by a small incision for outflow, the kidneys were preserved by in vivo perfusion fixation through the abdominal aorta. The kidneys were first perfused briefly with phosphate-buffered saline (PBS) to rinse away all blood and, subsequently, with 4% paraformaldehyde for 4 min.

Functional Data
Intact PTH was measured by an immunoradiometric assay specific for intact rat PTH (Nichols Institute, San Juan Capistrano, CA). Intra- and interassay coefficients of variation were 8.4 and 11.0%, respectively. VitD was extracted from plasma with diethyl ether, and extracts were chromatographed as described by Reinhardt et al. (22). The plasma VitD level was measured by a competitive protein assay using calf thymus as the source of binding protein. Plasma sodium was measured by a flame photometer (Instrumentation Laboratories, Lexington, MA). Serum blood urea nitrogen, creatinine, magnesium, calcium, and phosphorus were analyzed on the Ektachem 400 device (Eastman Kodak, Rochester, NY). Plasma CsA concentrations were measured by RIA (Incstar, Stillwater, MN).

Immunohistochemistry
Perfused kidneys were removed and fixed additionally by immersion in the same fixative solution for 2 h at 4°C. Slices of kidney tissue were dehydrated and embedded in polyester wax, and sections were cut and mounted on gelatin-coated glass slides. The sections were dewaxed with xylene and ethanol, and, after rinsing in tap water, sections were treated with methanolic H2O2 for 30 min. Before incubation with primary antibody, the sections were permeabilized by incubation for 15 min in 0.5% Triton X-100 in PBS and, subsequently, blocked with normal goat serum diluted 1:10 in PBS for 15 min, and incubated overnight at 4°C in the mouse antisemur against calbindin D28K (Sigma, St. Louis, MO) diluted 1:10,000 in PBS or Na-K-ATPase α1 subtypes (Upstate Biotechnology, Lake Placid, NY) diluted 1:00 in PBS. The sections were rinsed in PBS and incubated for 2 h in peroxidase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) or donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories). Sections were then incubated with the peroxidase-substrate solution, a mixture of 0.05% 3,3′-diaminobenzidine and 0.01% H2O2 for 5 min at room temperature. After being rinsed with Tris-HCl buffer, the sections were counterstained with hematoxylin and examined with light microscopy.

Western Blot Analysis
For Western blot analysis, renal cortex was homogenized in lysis buffer containing 20 mM Tris-HCl, 1% Triton X-100, 150 mM sodium chloride, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 0.02% sodium azide, 1 mM ethylene diamine tetra-acetic acid, 10 μM leupeptin, and 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 3000 × g for 20 min at 4°C. After determination of protein concentration in the supernatant by the Coomassie method (Pierce, Rockford, IL), samples were electrophoresed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis in reducing condition. Proteins were then transferred to nitrocellullose membranes by electroblocting. To reduce nonspecific antibody binding, the membranes were blocked with 5% nonfat dried milk for 30 min at room temperature, and then incubated for 24 h at 4°C with affinity-purified anti-calbindin D28K (1:50,000). The membranes were then washed in several changes of blotting buffer containing 0.01 M PBS, pH 7.4, and 0.1% Tween 20, and incubated for 1 h with peroxidase-labeled donkey anti-mouse IgG (1:1000, Jackson Immunoresearch Laboratories). Visualization was made after a 10- to 30-min exposure to enhanced chemiluminescence (Amersham Life Science, Buckinghamshire, United Kingdom) at room temperature. Densitometry analysis was performed using the Zero-Dscan software of Eagle EYETMII Still Video System (Stratagene, La Jolla, CA). The optical densities (mean ± SD) were obtained after three determinations for each band.

Statistical Analyses
Results are presented as mean ± SEM, and all statistical analyses were calculated with Statistical Package for Social Sciences version 6.1.1 for Macintosh. Comparisons between groups were done by unpaired t test. The level of statistical significance was chosen as P < 0.05.

Results
Functional Studies
Baseline parameters were not statistically different among the four groups (data not shown). Table 1 shows the body weight and biochemical parameters in experimental groups. Weight gains were progressive in the VH and CsA groups without significant difference throughout this experiment. Significant hypomagnesemia and inappropriately high fractional excretion of magnesium (FEmagnesia) was observed in the CsA group, suggesting renal magnesium wasting (P < 0.05 versus VH) (Table 1). Blood urea nitrogen was significantly increased in the CsA group compared with the VH group (P < 0.05). The CsA group showed a significant decrease in serum calcium
level (2.09 ± 0.07 versus 2.70 ± 0.12 mmol/L, P < 0.05) and an increase in urinary calcium excretion (0.028 ± 0.010 versus 0.017 ± 0.006 mmol/24 h, P < 0.05) compared with the VH group. The serum VitD and serum PTH levels were not significantly different between VH and CsA groups (43.3 ± 5.6 versus 45.6 ± 8.3 pg/ml and 14.7 ± 2.0 versus 15.6 ± 3.5 pg/ml, respectively) (Figure 1).

Animals treated with VitD and CsA showed severe weight loss compared with the VitD group (P < 0.05) despite pair feeding (Table 1). The VitD + CsA group showed a significant increase in blood urea nitrogen and serum creatinine compared with the VitD group (P < 0.05). The VitD + CsA group showed decreased serum calcium (2.84 ± 0.17 versus 3.26 ± 0.22 mmol/L, P < 0.05) and increased urinary calcium excretion (0.137 ± 0.044 versus 0.083 ± 0.012 mmol/24 h, P < 0.05) compared with the VitD group. There were no significant differences of serum VitD and PTH levels between VitD + CsA and VitD groups (195 ± 8 versus 201 ± 7 pg/ml and 4.5 ± 2.7 versus 5.7 ± 3.1 pg/ml, respectively) (Figure 2).

**Immunohistochemistry**

The intensity of the immunoreactivity of calbindin D_{28K} in renal tubules in the four groups is summarized in Table 2. The immunoreactivity of calbindin D_{28K} in the VH group was detected in the outer cortex, where it was localized in distal convoluted tubules and connecting tubules (Figure 3A). Immunostaining was much stronger in the connecting tubules than in the distal convoluted tubules (Figure 3B). The CsA group showed a decreased distribution of calbindin D_{28K} immunoreactivity compared with the VH group (Figure 4A). In the distal convoluted tubules, immunoreactivity of calbindin D_{28K} was almost undetectable, and connecting tubules showed weak immunoreactivity for calbindin D_{28K} compared with the VH group (Figure 4B).

Exogenous administration of VitD (VitD group) increased the overall distribution of calbindin D_{28K} immunoreactivity (Figure 5A). Immunoreactivity in the distal convoluted and connecting tubules was markedly increased compared with the VH group (Figure 5B). Immunoreactivity of calbindin D_{28K} was also observed in the cortical collecting tubules (Figure 5B). However, combined treatment with VitD and CsA decreased the overall distribution of calbindin D_{28K} compared with the VitD group (Figure 6A). Both the distal convoluted and connecting tubules showed weak immunoreactivity compared with the VitD group, and no immunoreactivity was detected in the collecting duct (Figure 6B).

To determine whether the observed effect of CsA was specific for calbindin D_{28K}, we also examined the immunostaining for Na-K-ATPase in the four experimental groups (Figure 7). In the VH group, immunoreactivity of Na-K-ATPase was detected in the distal convoluted tubules and thick ascending limb (Figure 7, A and B). The CsA group showed no significant change of Na-K-ATPase immunoreactivity compared with the VH group (Panels C and D). Exogenous administration of VitD increased the immunoreactivity of Na-K-ATPase in distal convoluted tubules and thick ascending limb compared with the VH group (Panels E and F). But the treatment with VitD

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**Table 1.** Body weight and biochemical parameters

<table>
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<th>Parameter</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
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<tr>
<td></td>
<td>VH (n = 7)</td>
<td>CsA (n = 7)</td>
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<tr>
<td>Body weight (g)</td>
<td>215 ± 10</td>
<td>210 ± 12</td>
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<tr>
<td>Albumin (g/dl)</td>
<td>3.6 ± 0.2</td>
<td>3.5 ± 0.1</td>
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<tr>
<td>Phosphorus (mg/dl)</td>
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<td>6.9 ± 0.6</td>
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<td>Magnesium (mg/dl)</td>
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<td>1.2 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>FE&lt;sub&gt;Mg&lt;/sub&gt; (%)</td>
<td>5.2 ± 0.9</td>
<td>11.7 ± 1.1&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>BUN (mg/dl)</td>
<td>16.8 ± 2.3</td>
<td>31.7 ± 3.0&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>0.47 ± 0.08</td>
<td>0.47 ± 0.08</td>
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<tr>
<td>Sodium (mEq/L)</td>
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<td>137 ± 3</td>
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<tr>
<td>FE&lt;sub&gt;Na&lt;/sub&gt; (%)</td>
<td>0.05 ± 0.01</td>
<td>0.06 ± 0.01</td>
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<tr>
<td>CsA conc. (mg/ml)</td>
<td>3450 ± 230</td>
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<sup>a</sup> VH, vehicle; CsA, cyclosporin A; VitD, 1,25-dihydroxyvitaminD<sub>3</sub>; FE<sub>Mg</sub>, fractional excretion of magnesium; BUN, blood urea nitrogen; FE<sub>Na</sub>, fractional excretion of sodium; conc., concentration.<br><br> <sup>b</sup> P < 0.05 compared with VitD group.<br><br> <sup>c</sup> P < 0.05 compared with VH group.
and CsA (VitD + CsA group) did not show any significant difference of the overall distribution and immunoreactivity of Na-K-ATPase compared with the VitD group (Panels G and H).

**Western Blot Analysis**

Immunoblot and relative optical densities of calbindin D$_{28K}$ are shown in Figure 8. Western blot analysis against calbindin D$_{28K}$ showed a single band at 28 kD (lane 1), which was decreased in CsA-treated rats (lane 2). When relative optical densities of the calbindin D$_{28K}$ band in each lane were compared using normal adult cortex as 100% reference, exogenous VitD (VitD group; lane 3) treatment increased the calbindin D$_{28K}$ protein significantly compared with the VH group (130.3% versus 100%, P < 0.05). In the CsA group (lane 2), calbindin D$_{28K}$ protein was markedly decreased compared with the VH group (23.5% versus 100%, P < 0.05). A comparison between the VitD + CsA and VitD groups revealed similar results (61.5% versus 130.3%, P < 0.05).

**Discussion**

Our study demonstrates an association between urinary calcium excretion and calbindin D$_{28K}$ expression in the CsA-treated rat. In rats that received CsA, there was a decrease in serum calcium concentrations, an increase in urinary calcium excretion, and a decrease in calbindin D$_{28K}$ protein levels and immunoreactivity compared with the VH group. In animals that received VitD, the addition of CsA resulted in a decrement in serum calcium concentration, an increase in urinary calcium excretion, and a decrease in calbindin D$_{28K}$ protein level and immunoreactivity when compared with animals that had received VitD alone. These results demonstrate an association of calbindin D$_{28K}$ expression with renal calcium handling in CsA-treated rats, and suggests that CsA-mediated downregulation of calbindin D$_{28K}$ might be a critical factor in the renal calcium wasting.

Until now, little was known about the association of CsA and calbindin D$_{28K}$ expression. Recently, Steiner et al. (19) reported decreased levels of calbindin D$_{28K}$ in kidneys of long-term CsA-treated rats, using enzyme-linked immunosorbent assay and two-dimensional protein electrophoresis. These investigators also reported an association of calbindin D$_{28K}$ expression with hypercalciuria in CsA-treated rats (20). However, their study did not examine the localization of calbindin D$_{28K}$ in kidney tissue, and did not provide data on the circulating concentration of VitD and PTH, which are involved in calcium regulation. In the present study, the influence of CsA...
on calbindin D28K expression and renal calcium handling was examined using four different approaches. First, calbindin D28K localization and calbindin D28K protein amount were studied by immunohistochemistry and Western blot analysis. Second, the serum VitD and PTH levels were measured to evaluate the association of these hormones with CsA. Third, calbindin D28K and Na-K-ATPase expressions were compared to evaluate the specific action of CsA on calbindin D28K. Fourth, the effect of CsA on calbindin D28K expression was observed at both basal and VitD-stimulated states to better understand the inhibitory action of CsA on calbindin D28K expression.

Previous studies have demonstrated calbindin D28K in the distal convoluted tubule and in the connecting tubule (12,13), where it is evenly distributed through the cytosol. In this study, the VH group showed strong immunostaining for calbindin D28K in the connecting tubule and weaker labeling of the distal convoluted tubule. However, CsA treatment decreased the overall distribution and intensity of calbindin D28K immunoreactivity, which was almost undetectable in the distal convoluted tubules and weak in the connecting tubules compared with the VH group. In the VitD group, overall immunoreactivity for calbindin D28K was markedly increased, and its expression was extended to the cortical collecting duct. This immunohistochemical study shows the inhibitory effect of CsA on calbindin D28K expression in the distal convoluted and connecting tubules, and stimulatory effect of VitD treatment on calbindin D28K expression in different nephron segments.

Microperfusion experiments support an active role of PTH in the fine-tuning of tubular calcium reabsorption, which takes place in segments of the distal nephron (23). Therefore, we measured the serum PTH levels for a possible link between calbindin D28K and serum PTH in CsA-treated rats. However, there was no significant difference in serum PTH levels between the VH and CsA groups. This finding suggests that short-term treatment of CsA does not affect the serum PTH levels. In addition to PTH, a relationship between CsA and VitD has been reported, although the results are conflicting (24–28). In general, low-dose CsA (5 mg/kg per d in humans) does not affect the circulating VitD levels, whereas a moderate dose of CsA (15 mg/kg per d) increases VitD levels in rat and humans by stimulating 1-α hydroxylase synthesis (28,29). In this study, CsA dose (25 mg/kg per d) was larger than in previous studies (28,29) on the presumption that CsA might decrease the calbindin D28K immunoreactivity by inhibiting 1-α hydroxylase synthesis in the proximal convoluted tubules.

Figure 2. Serum calcium, 24-h urinary calcium excretion, serum PTH, and VitD concentrations in VitD and VitD + CsA groups. *P < 0.05 compared with VitD group.
Figure 3. Immunolocalization of calbindin D<sub>28K</sub> protein in the VH group. (A) At low magnification, positive tubular profiles are seen mainly in the cortical labyrinth. (B) Immunostaining for calbindin D<sub>28K</sub> was weak in distal convoluted tubules (stars) and heavy in connecting tubules (CNT). Arrows indicate the intercalated cells with no immunoreactivity. Magnification: ×66 in A; ×400 in B.

Figure 4. Immunolocalization of calbindin D<sub>28K</sub> protein in the CsA group. (A) Positive tubular profiles were significantly decreased in the cortical labyrinth compared with the VH group. (B) Intensity of calbindin D<sub>28K</sub> immunoreactivity was markedly decreased in distal convoluted tubules (stars) and connecting tubules (CNT) compared with the VH group. Arrows indicate the negative intercalated cells. Magnification: ×66 in A; ×400 in B.
Figure 5. Immunolocalization of calbindin D$_{28K}$ protein in the VitD group. (A) Overall distribution of calbindin D$_{28K}$-positive tubular profiles was increased compared with VH group. In addition to distal convoluted tubules and connecting tubules, cortical collecting ducts (arrowheads) in medullary rays were also positive for calbindin D$_{28K}$. (B) Intensity of calbindin D$_{28K}$ immunoreactivity is markedly increased in distal convoluted tubules (stars) and connecting tubules (CNT) compared with the VH group. Arrows indicate the negative intercalated cells. Magnification: ×66 in A; ×400 in B.

Figure 6. Immunolocalization of calbindin D$_{28K}$ protein in the VitD + CsA group. (A) Overall distribution of calbindin D$_{28K}$ was markedly decreased compared with VitD group. (B) Intensity of calbindin D$_{28K}$ immunoreactivity also decreased significantly both in distal convoluted tubules (stars) and in connecting tubules (CNT) compared with VitD group. Arrows indicate the negative intercalated cells. Magnification: ×66 in A; ×400 in B.
Figure 7. Immunolocalization of Na-K-ATPase in cortex (A, C, E, and G) and inner stripe of outer medulla (B, D, F, and H) of VH (A and B), CsA (C and D), VitD (E and F), and VitD + CsA (G and H) groups. Positive immunoreactivity of Na-K-ATPase in the VH group was observed in the distal convoluted tubules (stars) and thick ascending limb (asterisks). In the CsA group, there was no significant change of immunoreactivity in both nephron segments (C and D) compared with VH group. In the VitD group, immunoreactivity of Na-K-ATPase was increased significantly in the distal convoluted tubules (stars) and thick ascending limb (asterisks) compared with VH group, but there was no difference of immunoreactivity between VitD and VitD + CsA groups (G and H). Magnification: ×330 in A through H.
CsA-treated rats, the expression of calbindin D$_{28K}$ (lane 2) was cortex treated with VH (lane 1) as 100% reference. In the kidney of the distal tubules and connecting segments (30). Our study shows that decreased calbindin D$_{28K}$ expression by CsA is not due to a nonspecific toxic effect in the distal convoluted tubule and connecting tubule. One interesting finding in this study is that VitD has a stimulatory effect not only on calbindin D$_{28K}$ expression, but also on Na-K-ATPase expression. This finding is consistent with previous reports that VitD stimulates the Na-K-ATPase activity in chondrocyte (31) and small intestine (32,33).

One of the biological effects of VitD action is the stimulation of biosynthesis of calbindin D$_{28K}$ in the distal convoluted tubule and connecting tubule (16–18). On the basis of these reports, we examined the effect of CsA on calbindin D$_{28K}$ expression with or without VitD treatment. In our study, CsA treatment decreased calbindin D$_{28K}$ expression significantly compared with the VH group (23.5% versus 100%), and the combined treatment of VitD and CsA resulted in a significant decrease in calbindin D$_{28K}$ expression compared with VitD alone (61.5% versus 130.3%). This observation indicates that CsA has an inhibitory action on calbindin D$_{28K}$ expression in VitD-stimulated states, as well as under normal conditions. However, immunohistochemistry still shows a larger increase and Western blot shows a greater band intensity in the VitD + CsA group than CsA group, suggesting incomplete inhibition of calbindin D$_{28K}$ by CsA in the VitD-stimulated state. Whether CsA can completely inhibit the VitD-stimulated calbindin D$_{28K}$ expression remains to be established. However, the results of our immunohistochemical studies indicate that CsA treatment completely prevents VitD-induced expression of calbindin D$_{28K}$ in the collecting duct.

In this study, significant hypomagnesemia was observed with CsA treatment, but hypocalcemia was marginal, albeit statistically significant. The hypomagnesemia caused by CsA is usually thought to be associated with a physiologic defect in the mTAL, which is the most vulnerable to CsA (34,35) and the major site for renal magnesium reabsorption (60% of the filtered load). In contrast, marginal hypocalcemia caused by CsA seems to be related to the functional defect of the distal convoluted tubule, which is less vulnerable to CsA and the minor site for renal calcium reabsorption (36). Therefore, the discrepancy between renal magnesium and calcium wasting after administration of CsA may be related to the different sites of reabsorption of the two ions and the preferential site of action of CsA.

It is difficult to evaluate the effect of CsA on renal calcium handling in a clinical setting. Hypocalcemia in renal transplant recipients might be caused by several pathogenetic factors (e.g., hypoalbuminemia, decreased production of calcitriol, hypomagnesemia, immunosuppressive drugs [steroids], volume status, and various clinical events [ischemia and rejection]) (37). Most clinical studies have focused on hyperparathyroidism and steroid use as the causes of bone and mineral disorders after renal transplantation (38). Little is known about the effect of CsA on bone turnover and renal calcium handling.
Inhibition of Calbindin D28K Expression by CsA

CsA-induced renal calcium wasting might be one of the causes of hypocalcemia after renal transplantation. In summary, the results of our study showed decreased calbindin D28K expression in distal convoluted and connecting tubules of rats with acute CsA nephrotoxicity. This finding was closely related to a decrease in serum calcium level and an increase in urinary calcium excretion, but was not associated with changes in circulating PTH or VitD. This observation suggests that decreased calbindin D28K expression in the distal tubule may play a role in renal calcium wasting after CsA administration.

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References


