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22-OXACALCITRIOL SUPPRESSES 25-HYDROXYCHOLECALCIFEROL-1α-HYDROXYLASE IN RAT KIDNEY

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Summary - 22-oxacalcitriol can decrease the serum level of 1,25-dihydroxycholecalciferol by increasing its metabolic clearance rate and decreasing its production rate. To determine whether 22-oxacalcitriol suppressed the renal 25-hydroxycholecalciferol-1α-hydroxylase we treated rats with 200 ng of 22-oxacalcitriol daily for 1 week. Enzyme activity was measured in vitro by measurement of production of 1,25-dihydroxycholecalciferol by renal slices incubated with 25-hydroxycholecalciferol. 22-oxacalcitriol significantly decreased the 25-hydroxycholecalciferol-1α-hydroxylase activity from 797 ± 208 pg of 1,25-dihydroxycholecalciferol/ g of kidney/ h in the control group to 257 ± 150 pg of 1,25-dihydroxycholecalciferol/ g of kidney/ h ($p < 0.05$). This is the first demonstration of suppression of the renal 25-hydroxycholecalciferol-1α-hydroxylase by the vitamin D_3 analog, 22-oxacalcitriol.

$1,25\text{-}(OH)_2D_3$ is the active metabolite of vitamin D_3 which under normal physiological conditions is synthesized in the renal cortex by a mitochondrial enzyme, $25\text{-}(OH)D_3$-1α-hydroxylase (1,2). There is evidence of synthesis of $1,25\text{-}(OH)_2D_3$ by extrarenal sites in conditions such as pregnancy, chronic renal failure, granulomatous disease, and lymphoma (3). The enzymes which catalyze the conversion of $25\text{-}(OH)D_3$ to $1,25\text{-}(OH)_2D_3$ in such states have not been fully characterized.

OCT, a vitamin D_3 analog with low calcemic activity, can induce cellular differentiation in vitro (4), inhibit growth of breast cancer in vitro and in vivo (5) and suppress parathyroid hormone secretion in vivo (6). In spite of this low calcemic activity, Dusso et al. (7) have demonstrated that OCT can decrease the serum level of $1,25\text{-}(OH)_2D_3$ by increasing its metabolic

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Abbreviations: $1,25\text{-}(OH)_2D_3$, 1,25-dihydroxycholecalciferol; $25\text{-}(OH)D_3$, 25-hydroxycholecalciferol; OCT, 22-oxacalcitriol; PTH, parathyroid hormone.

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clearance rate and decreasing its production rate. These changes are independent of changes in plasma calcium, phosphorous, and PTH. It was not clear whether OCT suppresses synthesis of 1,25-$(OH)_2D_3$ at the level of the kidney, extrarenal sites, or both. This is the first report demonstrating that OCT decreases the production of 1,25-$(OH)_2D_3$ by suppressing the rat renal 25-$(OH)D_3$-1α-hydroxylase in a normal physiological state.

MATERIALS AND METHODS

Materials: 25-$(OH)D_3$ and 1,25-$(OH)_2D_3$ were provided by Dr. Milan Uskokovic (Hoffman-LaRoche, Nutley, NJ). 1,25-dihydroxy[26,27-methyl-^{3}H]cholecalciferol (specific activity 130-180 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). OCT was synthesized by Chugai Pharmaceuticals (Tokyo, Japan).

Animal Protocol: Fischer-344 male rats (200-250 g) were purchased from Sasco, Inc. (Omaha, NE) and were maintained on Purina rat chow (0.9% calcium, 0.5% phosphorous) (Purina rat chow #5001, Ralston Purina, St. Louis, MO). 200 ng of OCT was administered daily intraperitoneally in 200 μl of propylene glycol for 7 days. Control rats recieved daily intraperitoneal injections of vehicle. All rats were sacrificed 24 h after the last injection.

Chemical Determinations: Plasma calcium was determined at 0, 3, 5, 7, 9, 11, and 24 h (at sacrifice) after the seventh dose of OCT. Plasma calcium was measured by atomic absorption spectrometry (Model 1100B, Perkin-Elmer Corp., Instrument Division, Norwalk, CT). Plasma phosphorous and creatinine were measured at sacrifice by Multistat III plus (Instrumentation Laboratory, Lexington, MA). Serum 1,25-$(OH)_2D_3$ was quantified by the extraction procedure of Hollis (8) and calf thymus receptor assay of Reinhardt et al. (9). As previously reported the contribution of OCT to the total measured 1,25-$(OH)_2D_3$ is negligible 24 h after the dose (7).

Production of 1,25-$(OH)_2D_3$: 24 h after the last dose of OCT the rats were anesthetized with 360 mg/kg of chloral hydrate. The rats were then exsanguinated and serum was analyzed for plasma calcium, phosphorous, and serum 1,25-$(OH)_2D_3$. The kidneys were quickly removed and placed in ice cold phosphate-buffered saline. Kidney cortical slices were prepared and incubated in 1.7 μM 25$(OH)D_3$ as described previously by Gray and Napoli (10) for periods of 0, 30 or 60 min. The reaction was stopped by quick-freezing the flasks in liquid nitrogen. The flasks were then stored in a -20 C freezer for later extraction of 1,25-$(OH)_2D_3$ and analysis. After thawing, 1400 dpm of 1,25$(OH)_2[^3H]D_3$ was added to each flask to monitor recovery. One volume of acetonitrile was added to each flask and the samples were homogenized with a Polytron (Brinkmann Instruments, Westbury, NJ) for 30 s. Samples were kept on ice for 40 minutes with occasional vortexing and then centrifuged at 1800 g for 15 min. The supernatant was applied directly to a prewashed PrepSep-C18 column (Fisher Scientific, Fair Lawn, NJ) which was subsequently washed with 5 ml of water. The fraction containing 1,25-$(OH)_2D_3$ was eluted with 4 ml of acetonitrile and dried under nitrogen. The samples were purified by HPLC with solvent composed of 3% isopropyl alcohol in methylene chloride. Recovery of added 1,25$(OH)_2[^3H]D_3$ through the extraction and purification procedure averaged 39 $\pm$ 1%. 1,25-$(OH)_2D_3$ determinations were performed using the calf thymus receptor assay of Reinhardt et al. (9).

Statistical Analysis: Results are expressed as mean $\pm$ SE. Nonpaired t tests were used to compare the control versus OCT treated groups. Paired t tests were used to compare the chemical determinations done on the same group over time.

RESULTS AND DISCUSSION

Table 1 reveals that the control and the OCT treated groups were similar with respect to baseline weight, plasma creatinine and phosphorous. OCT significantly decreased the serum

TABLE 1
BODY WEIGHT AND BLOOD CHEMISTRY OF CONTROL AND OCT TREATED RATS

	Control Rats	OCT Treated Rats
Weight (g)	275 ± 6	277 ± 4
Creatinine (mg/dl)	0.53 ± 0.03	0.52 ± 0.02
Plasma Phosphorous (mg/dl)	5.6 ± 0.2	5.8 ± 0.1
Plasma Calcium (mg/dl)	10.95 ± 0.13	11.06 ± 0.14
Serum 1,25-$(OH)_2D_3$ (pg/ml)	56.0 ± 6.9	14.9 ± 1.4*

* $p < 0.001$ versus control rats.

1,25-$(OH)_2D_3$ from 56.0 ± 6.9 pg/ml in the control group to 14.9 ± 1.4 pg/ml in the OCT treated group ($p < 0.001$) confirming the prior findings of Dusso et al. (7). The time course of production of 1,25-$(OH)_2D_3$ by kidney slices from control and OCT treated rats is shown in figure 1. Production of 1,25-$(OH)_2D_3$ was constant for at least 60 min. At time = 0 the endogenous tissue concentration of 1,25-$(OH)_2D_3$ was significantly greater in the control rats than in the OCT treated rats, 217 ± 45 pg of 1,25-$(OH)_2$D3/ g of kidney versus 92 ± 29 pg of 1,25-$(OH)_2$D3/ g of kidney ($p < 0.05$). Therefore, the production rate of 1,25-$(OH)_2D_3$ per hour was calculated by subtracting the t=0 tissue concentration from the 1,25-$(OH)_2$D3 tissue concentration measured at 1 hour. OCT significantly ($p < 0.05$) decreased the synthesis of

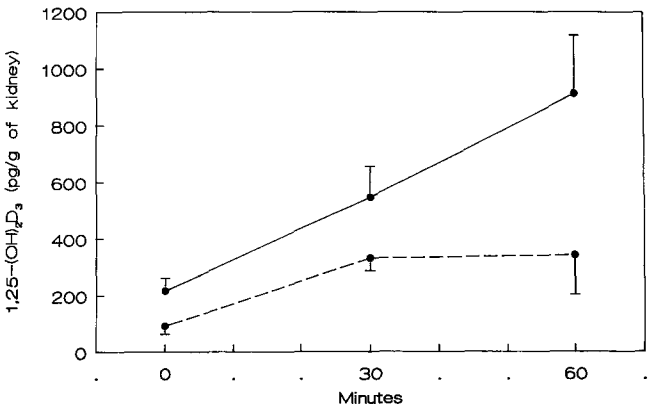

Figure 1. **Time course of 1,25-$(OH)_2D_3$ production from kidney slices.** The slices were incubated with 1.7 μM 25$(OH)D_3$ for 0, 30 or 60 minutes. Data points represent the mean ± SE in OCT treated rats (-----) compared to controls (__). 1,25-$(OH)_2D_3$ determinations from the kidney slices from OCT treated rats were significantly different from controls at all time points ($p < 0.05$).

$1,25\text{-}(OH)_2D_3$ from 797 $\pm$ 208 pg of $1,25\text{-}(OH)_2D_3$/ g of kidney/ h in the control group (n = 7) to 257 $\pm$ 150 pg of $1,25\text{-}(OH)_2D3$/ g of kidney/ h in the OCT treated group (n = 8). This decrease in $1,25\text{-}(OH)_2D_3$ production demonstrates suppression of the renal $25\text{-}(OH)D_3\text{-}1\alpha$-hydroxylase by OCT.

The exact mechanism for the suppression of the renal $25\text{-}(OH)D_3\text{-}1\alpha$-hydroxylase by OCT is not known. The activity of the renal $25\text{-}(OH)D_3\text{-}1\alpha$-hydroxylase is regulated by many factors including calcium, phosphorous, $1,25\text{-}(OH)_2D_3$, PTH, calcitonin, growth hormone, insulin, and metabolic acidosis (11,12,13,14). Although, like $1,25\text{-}(OH)_2D_3$, OCT may be directly suppressing expression of the renal $25\text{-}(OH)D_3\text{-}1\alpha$-hydroxylase it is also possible that changes in PTH and calcium may be mediating the effect of OCT on this enzyme. Since the serum concentration of OCT is negligible 24 h after administration it is unlikely to be directly inhibiting the enzyme at that time. In the same studies we have also shown that serum $1,25\text{-}(OH)_2D_3$ returns to basal levels 96 h after the last dose of OCT (7). This implies a genomic effect of OCT on the expression of the enzyme itself. Prior studies by Dusso et al. have demonstrated that this effect of OCT on serum $1,25\text{-}(OH)_2D_3$ is independent of calcium and phosphorous (7). Brown et al. have shown that OCT is not calcemic at doses greater than that used in this experiment 24 h after administration (6). Neither of these studies excluded a transient rise in serum calcium following a dose of OCT. We measured plasma calcium at several times during the 24 hours after administration of the last dose of OCT. Figure 2 shows that OCT did not produce any increase in plasma calcium. The plasma calcium in the OCT-treated rats were slightly higher at 9 and 11 h after the dose (10.47 $\pm$ 0.10 mg/dl in the OCT treated group

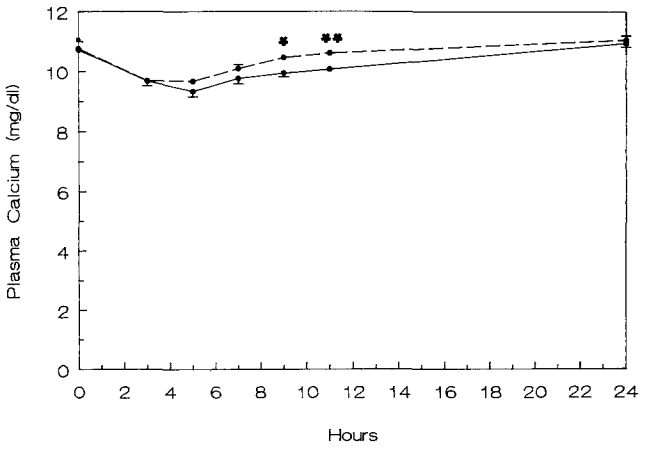

Figure 2. **Time course of plasma calcium after a 200 ng dose of OCT (-----) compared to vehicle treated rats (_).** Data points represent the mean $\pm$ SE. Significance between groups at specified time points was as follows:
*, $p < 0.05$ and **, $p < 0.001$.

versus 9.95 $\pm$ 0.13 mg/dl in the control group at 9 h ($p < 0.05$) and, respectively, 10.62 $\pm$ 0.05 mg/dl versus 10.08 $\pm$ 0.09 mg/dl at 11 h ($p < 0.001$)). It is unlikely that this small transient difference in plasma calcium contributes significantly to the greater than 50 percent suppression of renal 25-(OH)D_3-1α-hydroxylase activity. On the other hand, this dose of OCT has been shown to dramatically reduce PTH levels (6). Reduction of PTH in these rats probably plays a significant role in the decrease of 25-hydroxyvitamin D_3-1α-hydroxylase activity by OCT. However, Dusso et al. did show that OCT suppressed 25-(OH)D_3-1α-hydroxylase activity in parathyroidectomized rats (7). Thus, it is likely that OCT can suppress the renal enzyme activity independent of changes in PTH.

This is the first demonstration of suppression of the renal 25-(OH)D_3-1α-hydroxylase by a vitamin D_3 analog, OCT. Following the administration of OCT this decrease in 1,25-$(OH)_2D_3$ synthesis at the level of the kidney, accompanied by the previously demonstrated increase in metabolic clearance rate, results in the decrease in serum 1,25-$(OH)_2D_3$. This drop in 1,25-$(OH)_2D_3$ may contribute to the low calcemic activity of OCT.

Although OCT shares many of the functional properties of 1,25-$(OH)_2D_3$, the analog appears to be unable to support other actions of the natural hormone; for example, OCT cannot acutely increase intracellular calcium in osteogenic sarcoma ROS 17/2.8 cells (15) nor increase intracellular calcium and induce the respiratory burst necessary for antimicrobial and phagocytic activities in human leukemic HL-60 cells (16). The physiological effects of this and possibly other functional deficiencies of OCT are unclear. It is likely that the ability of OCT to inhibit tumor growth and suppress secondary hyperparathyroidism will be exploited in the treatment of disease and then the clinical relevance of these experimental differences will be determined.

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