0013-7227/93/1336-2719$03.00/0
Endocrinology

Vol. 133, No. 6
Printed in U.S.A.

# Differential Catabolism of 22-Oxacalcitriol and 1,25-Dihydroxyvitamin $D_3$ by Normal Human Peripheral Monocytes*

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**ABSTRACT**

22-Oxacalcitriol [1,25-$(OH)_2$-22oxa-$D_3$] mimics the action of 1,25-dihydroxyvitamin $D_3$ [1,25-$(OH)_2D_3$] in a variety of target tissues, including the systemic control of calcitriol metabolism. Similar to 1,25-$(OH)_2D_3$, 1,25-$(OH)_2$-22oxa-$D_3$ decreases the rate of 1,25-$(OH)_2D_3$ synthesis and accelerates its metabolic clearance rate. We have previously shown that in normal human monocytes, physiological concentrations of 1,25-$(OH)_2D_3$ and 1,25-$(OH)_2$-22oxa-$D_3$ determine identical suppression of 1,25-$(OH)_2D_3$ synthesis. Moreover, both sterols have a similar potency to induce vitamin D degradation through stimulation of the C24-hydroxylation pathway. In this study, we examined the ability of normal human monocytes to metabolize 1,25-$(OH)_2$-22oxa-$D_3$ and whether the enzymes involved are the same as those that catabolize 1,25-$(OH)_2D_3$. Time-course experiments demonstrated no detectable basal catabolic activity. However, exogenous 1,25-$(OH)_2D_3$ at physiological concentrations induced 1,25-$(OH)_2$-22oxa-$D_3$ degradation by normal human monocytes. Competition experiments showed that a 10-fold molar excess of unlabeled 1,25-$(OH)_2D_3$ inhibited tritiated-1,25-$(OH)_2$-22oxa-$D_3$ catabolism by 85%, whereas a 10-fold excess of unlabeled 1,25-$(OH)_2$-22oxa-$D_3$ reduced tritiated-1,25-$(OH)_2$-22oxa-$D_3$ catabolism by 33%. In contrast, although a 10-fold excess of unlabeled 1,25-$(OH)_2D_3$ reduced tritiated 1,25-$(OH)_2D_3$ catabolism by 60%, a 1000-fold excess of 1,25-$(OH)_2$-22oxa-$D_3$ was required to reduce tritiated 1,25-$(OH)_2D_3$ catabolism to this degree. The apparent $K_m$ for 1,25-$(OH)_2$-22oxa-$D_3$ was significantly higher than that of 1,25-$(OH)_2D_3$ ($2.0 \pm 0.8$ *vs.* $0.9 \pm 0.2$ nM, respectively; $P < 0.001$) for the catabolic pathway induced by physiological concentrations of 1,25-$(OH)_2D_3$. Moreover, the presence of 0.65 nM 1,25-$(OH)_2D_3$ caused an additional increase in the $K_m$ for 1,25-$(OH)_2$-22oxa-$D_3$ ($3.2 \pm 0.8$ nM). These data suggest that 1,25-$(OH)_2$-22oxa-$D_3$ may be less accessible than 1,25-$(OH)_2D_3$ to the hydroxylases involved in vitamin D catabolism. The resulting prolonged biological half-life of the analog in certain target tissues may be involved in its selectivity. (*Endocrinology* **133:** 2719–2723, 1993)

THE ENZYMATIC conversion of 25-hydroxyvitamin $D_3$ (25$OHD_3$) to 1,25-dihydroxyvitamin $D_3$ [1,25-$(OH)_2D_3$] is the most important step in vitamin D metabolism. Although 1,25-$(OH)_2D_3$ is produced primarily in the kidney, extrarenal 1,25-$(OH)_2D_3$ synthesis has been reported *in vitro* in placenta (1, 2), osteoclasts derived from bone marrow macrophages (3), keratinocytes (4, 5), and macrophages from sarcoid (6) and tuberculous granulomata (7). In bone marrow and alveolar macrophages from normal humans, 25-hydroxycholecalciferol 1$\alpha$-hydroxylase can be induced by treatment with $\gamma$-interferon or lipopolysaccharide (8–10). The high intracellular 1,25-$(OH)_2D_3$ levels in activated macrophages induce the production of 24,25-$(OH)_2D_3$ (10).

Our laboratory has demonstrated that monocytes derived from peripheral blood mononuclear leukocytes from normal subjects can convert 25$(OH)D_3$ to 1,25-$(OH)_2D_3$ (11). Exogenous 1,25-$(OH)_2D_3$ inhibits 1,25-$(OH)_2D_3$ synthesis and stimulates 1,25-$(OH)_2D_3$ catabolism in a dose-dependent fashion (11, 12). Four-hour exposure of normal human macrophages to a physiological (0.24 nM) concentration of the sterol reduces macrophage 1$\alpha$-hydroxylase activity and stimulates the synthesis of metabolites more polar than 1,25-$(OH)_2D_3$ (13).

In kidney and intestine, C-24 oxidation of 1,25-$(OH)_2D_3$ is the major metabolic pathway involved in the inactivation of circulating 1,25-$(OH)_2D_3$ (14, 15). Previous studies in our laboratory have demonstrated that in normal human peripheral monocytes (NHPM), exogenous 1,25-$(OH)_2D_3$ induces its own catabolism through activation of the C-24 hydroxylation pathway (13).

The vitamin D analog 22-oxacalcitriol [1,25-$(OH)_2$-22oxa-$D_3$] mimics 1,25-$(OH)_2D_3$ in the control of systemic calcitriol homeostasis. It inhibits 1,25-$(OH)_2D_3$ synthesis in rat kidney cells (16). Moreover, it has a potency similar to that of 1,25-$(OH)_2D_3$ to induce catabolism and suppress 1,25-$(OH)_2D_3$ generation by normal macrophages (13). On the other hand, 1,25-$(OH)_2$-22oxa-$D_3$ is 10 times more potent than 1,25-$(OH)_2D_3$ in suppressing cell growth and inducing differentiation of the mouse myelocytic leukemic cell line WEHI-3 (17, 18), and it enhances the immune response with a potency 50 times higher than 1,25-$(OH)_2D_3$ in mice (19). In psoriatic fibroblasts, 1,25-$(OH)_2$-22oxa-$D_3$ inhibited growth more effectively than 1,25-$(OH)_2D_3$ (20). On the contrary, 1,25-$(OH)_2$-22oxa-$D_3$ has much lesser direct effects on bone metabolism in normal male rats than 1,25-$(OH)_2D_3$ (21). Our

Received February 10, 1993.

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* This work was supported in part by USPHS NIDDK Grants DK-09976 and DK-07126 and a grant provided by Chugai Pharmaceuticals Co., Ltd. (Tokyo, Japan).

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laboratory has also demonstrated that the administration of 170 ng 1,25-$(OH)_2$-22oxa-$D_3$/100 g BW to rats for 4 days did not cause the hypercalcemia (22). The mechanisms responsible for the diverse biological activity are still controversial.

The affinity of 1,25-$(OH)_2$-22oxa-$D_3$ for the 1,25-$(OH)_2D_3$ receptor (VDR) in HL60 cells is one tenth that of 1,25-$(OH)_2D_3$ (17). However, the analog exhibits a more potent activity than 1,25-$(OH)_2D_3$ in inducing cell differentiation (20) and more enhanced immunoregulating effects (19). The affinities of the vitamin D analogs for VDR are, therefore, not necessarily correlated with their biological activities. It is possible that other factors, such as cellular uptake, intracellular metabolism, and, therefore, the biological half-life of vitamin D compounds in different target tissues, are important for their selectivity.

With regard to cellular uptake, it has been shown that the binding affinity of 1,25-$(OH)_2$-22oxa-$D_3$ for serum vitamin D-binding protein (DBP) is much lower than that of 1,25-$(OH)_2D_3$ (12, 19, 23). The lower affinity of 1,25-$(OH)_2D_3$-22oxa-$D_3$ for DBP results in a higher proportion of the free form of the sterol in the circulation (12); therefore, in the presence of serum, 1,25-$(OH)_2$-22oxa-$D_3$ is more effective than 1,25-$(OH)_2D_3$ in inducing vitamin D catabolism in normal macrophages (12).

The present studies were focused on intracellular metabolism. We compared the abilities of normal human monocytes to catabolize 1,25-$(OH)_2$-22oxa-$D_3$ and 1,25-$(OH)_2D_3$.

## Materials and Methods

### *Materials*

1,25-$(OH)_2D_3$ was kindly provided by Dr. Milan Uskokovic (Hoffman LaRoche, Nutley, NJ). 1,25-Dihydroxy[26,27-*methyl*-$^3$H]cholecalciferol (SA, 174 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). 1,25-$(OH)_2$-22oxa-$D_3$) and 22-oxa-[26-$^3$H]calcitriol (SA, 86.3 Ci/mmol) were synthesized by Chugai Pharmaceuticals (Tokyo, Japan).

### *Culture of human monocytes*

Peripheral blood was obtained from normal volunteers by venipuncture. Mononuclear leukocytes were isolated by Ficoll-Hypaque separation (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ). Cells were plated in six-well plates at a concentration of $7 \times 10^6$ cells/well in 1 ml RPMI-1640 with 1% fatty acid-free albumin, 50,000 U/liter penicillin G sodium, 50,000 μg/liter streptomycin sulfate, 10 mM HEPES, and 0.8 mM sodium bicarbonate. Cells were maintained at 37 C in a humidified atmosphere containing 95% air-5% $CO_2$. After an incubation period of 18 h, nonadherent cells and media were removed. More than 95% of the adherent cells stained positively for monocyte-macrophage-specific α-naphtyl acetate esterase activity. Adherent cells were washed once with PBS and twice with RPMI-1640 containing 0.1% fatty acid-free albumin. One milliliter of RPMI-1640 (0.1% fatty acid-free albumin) was then added to adherent cells. Assays for 1,25-$(OH)_2$-22oxa-$D_3$ or 1,25-$(OH)_2D_3$ catabolism were initiated by the addition of 0.26 nM [$^3$H]1,25-$(OH)_2$-22oxa-$D_3$ or [$^3$H]1,25-$(OH)_2D_3$, respectively. The reaction was stopped after 1 h of incubation at 37 C with 1 ml acetonitrile. To quantitate recoveries, 100 ng radioinert 1,25-$(OH)_2$-22oxa-$D_3$ or 1,25-$(OH)_2D_3$ were added to the samples for 1,25-$(OH)_2$-22oxa-$D_3$ and 1,25-$(OH)_2D_3$ catabolism experiments, respectively. Samples were extracted using $C_{18}$ cartridges (Fisher Scientific, Fair Lawn, NJ), as described by Reinhardt *et al.* (24), and further purified on normal phase HPLC (4.5 mm × 25 cm Zorbax-Sil; 4.5 μm; Phenomenex, Torrance, CA), using methylene chloride-isopropanol (97:3). One-milliliter fractions eluting with 1,25-$(OH)_2D_3$ or 1,25-$(OH)_2$-22oxa-$D_3$ were collected, dried, mixed with 3 ml Scintiverse BD (Fisher Scientific), and counted for tritium (ICN Micrometic System, Inc., Huntsville, AL). Counts per min were corrected for recovery using the area of UV absorption peak of the internal standard. Results were normalized to DNA content of cells, measured in the pellets of the extraction procedure using the ethidium bromide method (25), and expressed as femtomoles of metabolite per μg DNA.

### *Effect of exogenous 1,25-$(OH)_2D_3$ on the catabolism of 1,25-$(OH)_2$-22oxa-$D_3$ by normal human monocytes*

Exogenous 1,25-$(OH)_2D_3$, at a concentration of 0.24 nM, was added to peripheral blood mononuclear cells plated at a concentration of $7 \times 10^6$ cells in 1 ml RPMI-1640 containing 1% fatty acid-free albumin. Cells were incubated at 37 C for 18 h with or without 0.24 nM 1,25-$(OH)_2D_3$. Adherent cells were washed, and 0.26 nM [$^3$H]-1,25-$(OH)_2$-22oxa-$D_3$ was added in 1 ml medium. The amount of [$^3$H]1,25-$(OH)_2$-22oxa-$D_3$ remaining was measured, as indicated above, at 0, 0.5, 1, 2, 3, 4, 6, and 12 h. For each time point, determinations were performed in triplicate.

### *Competition experiments*

Competition experiments [tritiated 1,25-$(OH)_2D_3$ with radioinert 1,25-$(OH)_2$-22oxa-$D_3$ and tritiated 1,25-$(OH)_2$-22oxa-$D_3$ with radioinert 1,25-$(OH)_2D_3$] were carried out in peripheral blood mononuclear cells that had been pretreated for 18 h with 0.24 nM 1,25-$(OH)_2D_3$ to induce the catabolic pathway. The adherent cells were washed and incubated with 0.26 nM [$^3$H]1,25-$(OH)_2$-22oxa-$D_3$ alone or in the presence of 2.6 nM radioinert 1,25-$(OH)_2$-22oxa-$D_3$ or 1.3 or 2.6 nM radioinert 1,25-$(OH)_2D_3$. Catabolism of [$^3$H]1,25-$(OH)_2D_3$ (0.26 nM) was measured in the presence of 2.6 nM radioinert 1,25-$(OH)_2D_3$ or 2.6 or 260 nM radioinert 1,25-$(OH)_2$-22oxa-$D_3$. The reaction was stopped, as previously indicated, after 1 h of incubation at 37 C. The fraction coeluting with authentic 1,25-$(OH)_2D_3$ or 1,25-$(OH)_2$-22oxa-$D_3$ in normal phase HPLC was dried and counted for tritium. Determinations were performed in triplicate for each experimental condition, corrected per μg DNA in the sample, and expressed as femtomoles of metabolite per μg DNA. The percent 1,25-$(OH)_2D_3$ or 1,25-$(OH)_2$-22oxa-$D_3$ catabolized was calculated as follows: % of catabolism = $[(A - B)/A] \times 100$, where A represents femtomoles of [$^3$H]1,25-$(OH)_2D_3$ or 1,25-$(OH)_2$-22oxa-$D_3$ in control wells at time zero, and B represents femtomoles of 1,25-$(OH)_2D_3$ or 1,25-$(OH)_2$-22oxa-$D_3$ in experimental wells after 1-h incubation.

### *Kinetic analysis*

Four different substrate concentrations (0.13, 0.26, 0.52, and 0.78 nM) were assayed in triplicate to determine the $K_m$ for 1,25-$(OH)_2D_3$ and 1,25-$(OH)_2$-22oxa-$D_3$ and the respective maximal velocities ($V_{max}$) of the enzymes involved in vitamin D catabolism. $K_m$ and $V_{max}$ were obtained using the double reciprocal plot of Lineweaver and Burk with linear regression analysis of the data. The inhibitory effect of 1,25-$(OH)_2D_3$ on 1,25-$(OH)_2$-22oxa-$D_3$ catabolism was also tested using 0.13, 0.26, 0.52, and 0.78 nM tritiated 1,25-$(OH)_2$-22oxa-$D_3$ as substrate in the presence of 0.65 nM radioinert 1,25-$(OH)_2D_3$ in the incubation medium.

### *Statistical analysis*

Results are expressed as the mean ± SEM. Statistical evaluation of the data was performed by Student's *t* test for unpaired observations.

## Results

The time course for 1,25-$(OH)_2$-22oxa-$D_3$ catabolism is depicted in Fig. 1. Macrophages obtained from four normal volunteers were examined. No degradation of 1,25-$(OH)_2$-22oxa-$D_3$ could be detected in the first 2 h (Fig. 1, —). Thereafter, the amount of [$^3$H]1,25-$(OH)_2$-22oxa-$D_3$ remaining in the well decreased linearly. By 4 h, 57.8 ± 1.6% of

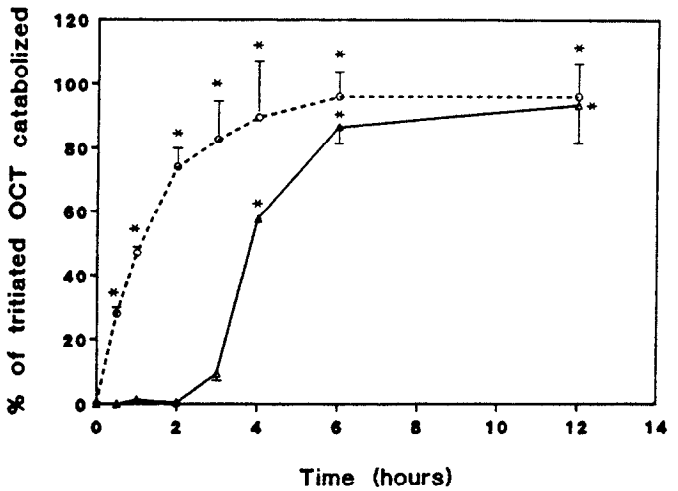


FIG. 1. Time course for 1,25-(OH)$_2$-22oxa-D$_3$ catabolism by normal human mononuclear leukocytes. Peripheral blood mononuclear cells were incubated in serum-free medium with (○) or without (△) exogenous 1,25-(OH)$_2$D$_3$ (0.24 nM) for 18 h. Nonadherent cells and media were removed. Adherent cells were incubated for the specified time with 0.26 nM tritiated 1,25-(OH)$_2$-22oxa-D$_3$. Remaining tritiated 1,25-(OH)$_2$-22oxa-D$_3$ was measured as outlined in *Materials and Methods*. Results are expressed as the percentage of 1,25-(OH)$_2$-22oxa-D$_3$ catabolized. Values are the mean and SEM of four independent experiments. Experiments were performed in triplicate. *, Differs from control (time zero), $P < 0.001$. OCT, 1,25-(OH)$_2$-22oxa-D$_3$.

1,25-(OH)$_2$-22oxa-D$_3$ had been catabolized. This suggests that 1,25-(OH)$_2$-22oxa-D$_3$ at 0.26 nM can induce its own catabolism through a genomic effect.

When the enzyme(s) responsible for vitamin D catabolism was induced by preincubation of NHPM with 0.24 nM 1,25-(OH)$_2$D$_3$ for 18 h, 1,25-(OH)$_2$-22oxa-D$_3$ catabolism was accelerated (Fig. 1, - - -). After 1-h incubation, 47.1 ± 0.9% (n = 4) of the 1,25-(OH)$_2$-22oxa-D$_3$ was catabolized, and more than 95% was degraded after 6 h.

To test whether 1,25-(OH)$_2$D$_3$ and 1,25-(OH)$_2$-22oxa-D$_3$ followed the same catabolic pathway, competition experiments were performed with cells from normal volunteers after induction of catabolic pathways by an 18-h exposure to 0.24 nM exogenous 1,25-(OH)$_2$D$_3$. Results of the experiments of 1,25-(OH)$_2$-22oxa-D$_3$ catabolism are shown in Fig. 2. Control cells were incubated with 0.26 nM [$^3$H]1,25-(OH)$_2$-22oxa-D$_3$, as indicated in *Materials and Methods*, and after 1 h, 44.8 ± 2.3% (n = 9) of the 1,25-(OH)$_2$-22oxa-D$_3$ had been catabolized ($P < 0.05$). Inclusion of 2.6 nM radioinert 1,25-(OH)$_2$-22oxa-D$_3$ reduced degradation of [$^3$H]1,25-(OH)$_2$-22oxaD$_3$ catabolism to 30.2 ± 1.7% (n = 5) during the 1-h incubation ($P < 0.01$). Competition with 2.6 nM 1,25-(OH)$_2$D$_3$ almost completely blocked the catabolism of [$^3$H]1,25-(OH)$_2$-22oxa-D$_3$(6.8 ± 0.2% degraded; n = 5; $P < 0.001$). Coincubation with 1.3 nM 1,25-(OH)$_2$D$_3$ for 1 h markedly inhibited 1,25-(OH)$_2$-22oxa-D$_3$ catabolism (19.2 ± 2.3% degraded; n = 5; $P < 0.01$).

The effect of radioinert 1,25-(OH)$_2$-22oxa-D$_3$ on the catabolism of [$^3$H]1,25-(OH)$_2$D$_3$ by NHPM is shown in Fig. 3. Experiments were performed after preincubation of peripheral blood mononuclear cells with 0.24 nM 1,25-(OH)$_2$D$_3$ for 18 h to induce catabolism. Adherent cells were incubated

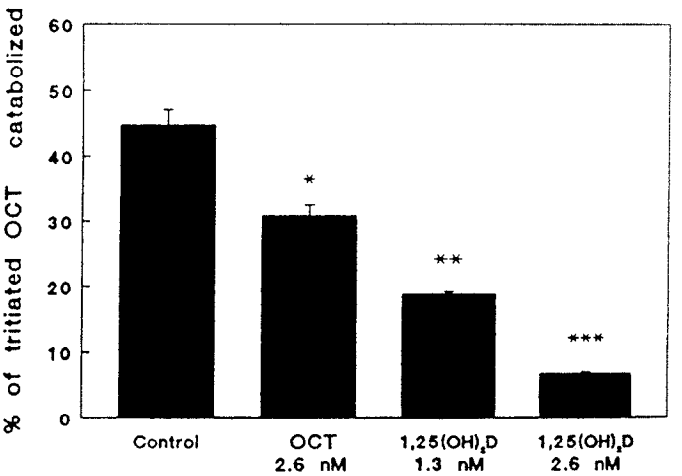


FIG. 2. Inhibition of tritiated 1,25-(OH)$_2$-22oxa-D$_3$ catabolism by radioinert 1,25-(OH)$_2$-22oxa-D$_3$ and 1,25-(OH)$_2$D$_3$. Peripheral blood mononuclear cells were incubated for 18 h with 0.24 nM radioinert 1,25-(OH)$_2$D$_3$. Nonadherent cells and media were removed. Adherent cells were incubated for 1 h with 0.26 nM tritiated 1,25-(OH)$_2$-22oxa-D$_3$ (control; n = 9) in the presence of 2.6 nM radioinert 1,25-(OH)$_2$-22oxa-D$_3$ (n = 5) or either 1.3 nM (n = 5) or 2.6 nM radioinert 1,25-(OH)$_2$D$_3$ (n = 5). The tritiated 1,25-(OH)$_2$-22oxa-D$_3$ remaining was measured as outlined in *Materials and Methods*. Results are expressed as the percentage of 1,25-(OH)$_2$-22oxa-D$_3$ catabolized in 1 h. Values are the mean and SEM. Experiments were performed in triplicate. *, Differs from control, $P < 0.001$. **, Differs from 1,25-(OH)$_2$-22oxa-D$_3$ (2.6 nM), $P < 0.01$. ***Differs from 1,25-(OH)$_2$-22oxa-D$_3$ (2.6 nM), $P < 0.001$. OCT, 1,25-(OH)$_2$-22oxa-D$_3$; 1,25-(OH)$_2$D, 1,25-(OH)$_2$D$_3$.

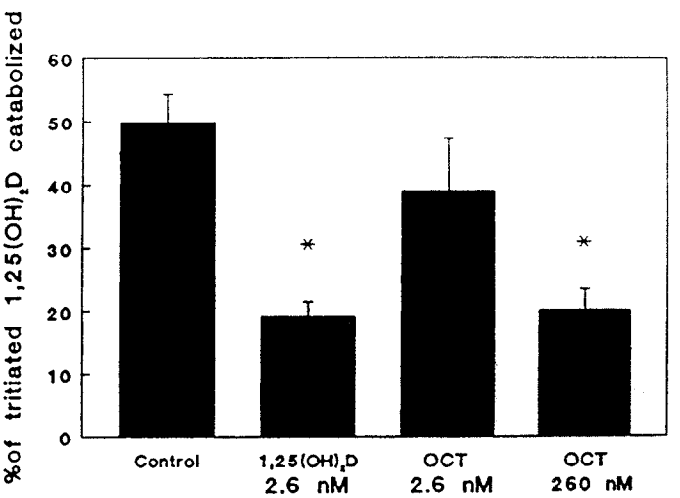


FIG. 3. Inhibition of tritiated 1,25-(OH)$_2$D$_3$ catabolism by radioinert 1,25-(OH)$_2$D$_3$ and 1,25-(OH)$_2$-22oxa-D$_3$. Peripheral blood mononuclear cells were incubated for 18 h with 0.24 nM radioinert 1,25-(OH)$_2$D$_3$. Nonadherent cells and media were removed. Adherent cells were incubated for 1 h with 0.26 nM tritiated 1,25-(OH)$_2$D$_3$ (control; n = 8) in the presence of 2.6 nM radioinert 1,25-(OH)$_2$D$_3$ (n = 4) or either 2.6 nM (n = 4) or 260 nM radioinert 1,25-(OH)$_2$-22oxa-D$_3$ (n = 4). The tritiated 1,25-(OH)$_2$D$_3$ remaining was measured as outlined in *Materials and Methods*. Results are expressed as the percentage of 1,25-(OH)$_2$D$_3$ catabolized in 1 h. Values are the mean and SEM. Experiments were performed in triplicate. *, Differs from control, $P < 0.01$. OCT, 1,25-(OH)$_2$-22oxa-D$_3$; 1,25-(OH)$_2$D, 1,25-(OH)$_2$D$_3$.

with 0.26 nM [$^3$H]1,25-(OH)$_2$D$_3$ for 1 h, as indicated in *Materials and Methods*. In controls, 49.9 ± 4.4% (n = 8) of the [$^3$H]1,25-(OH)$_2$D$_3$ was catabolized. Inclusion of 2.6 nM

radioinert 1,25-$(OH)_2D_3$ reduced catabolism to 18.9 ± 3.2% (n = 4) during 1-h incubation ($P < 0.01$). Inclusion of 2.6 nM 1,25-$(OH)_2$-22oxa-$D_3$ did not affect 1,25-$(OH)_2D_3$ catabolism (n = 4). A concentration of 1,25-$(OH)_2$-22oxa-$D_3$ (260 nM) 1000 times higher than that of the [$^3$H]1,25-$(OH)_2D_3$ reduced degradation by only 20.1 ± 3.3% (n = 4). These results suggested that the hydroxylases involved in the catabolic pathway stimulated by exogenous 1,25-$(OH)_2D_3$ in NHPM have a lower affinity for 1,25-$(OH)_2$-22oxa-$D_3$ than for 1,25-$(OH)_2D_3$.

Table 1 shows the kinetic parameters for the catabolism of 1,25-$(OH)_2D_3$ and 1,25-$(OH)_2$-22oxa-$D_3$ by NHPM. The $K_m$ for 1,25-$(OH)_2$-22oxa-$D_3$ of the enzymes of the catabolic pathway was significantly higher than that for 1,25-$(OH)_2D_3$ ($P < 0.001$). The $V_{max}$ of 1,25-$(OH)_2$-22oxa-$D_3$ was slightly, but significantly, higher than that of 1,25-$(OH)_2D_3$ ($P < 0.05$). We also examined $K_m$ and $V_{max}$ when NHPM were coincubated with [$^3$H]-1,25-$(OH)_2$-22oxa-$D_3$ (0.13, 0.26, 0.52, and 0.78 nM) and nonradioactive 1,25-$(OH)_2D_3$ (0.65 nM). The $K_m$ for 1,25-$(OH)_2$-22oxa-$D_3$ was further increased to 3.2 ± 0.8 nM (n = 5; $P < 0.001$), without significant changes in the $V_{max}$.

## Discussion

Our laboratory has demonstrated that 1,25-$(OH)_2$-22oxa-$D_3$ lowers serum 1,25-$(OH)_2D_3$ levels in normal rats by a mechanism independent of PTH suppression (26). 1,25-$(OH)_2$-22oxa-$D_3$ mimics 1,25-$(OH)_2D_3$ in the induction of the enzymatic processes involved in 1,25-$(OH)_2D_3$ degradation, increasing the MCR of the sterol. It also suppresses renal 1,25-$(OH)_2D_3$ synthesis in normal animals (16). We have reported that 0.24 nM 1,25-$(OH)_2$-22oxa-$D_3$ has a potency similar to that of 1,25-$(OH)_2D_3$ in inducing catabolism and suppressing 1,25-$(OH)_2D_3$ synthesis by normal human peripheral macrophages (13). Clearly, 1,25-$(OH)_2$-22oxa-$D_3$ mimics 1,25-$(OH)_2D_3$ in the control of systemic and extrarenal vitamin D metabolism. The lower affinity of 1,25-$(OH)_2$-22oxa-$D_3$ for DBP results in a higher proportion of the free active form in serum and a more rapid clearance from the circulation (12). The higher proportion of free 1,25-$(OH)_2$-22oxa-$D_3$ renders the analog more effective than 1,25-$(OH)_2D_3$ in stimulating vitamin D catabolism at various serum concentrations in NHPM (12). It is possible that preferential uptake of either the free or bound form by different target cells plays a role in the selectivity of 1,25-$(OH)_2$-22oxa-$D_3$. In the intracellular catabolism of 1,25-$(OH)_2D_3$, the prevalent pathway is C-24 oxidation (27–29), in which 1,25-$(OH)_2D_3$ is first hydroxylated at carbon 24 to render 1,24,25-$(OH)_2D_3$. Brown *et al.* (30) suggested that 1,25-$(OH)_2$-22oxa-$D_3$ is metabolized by a pathway that is probably identical to the vitamin D-inducible side-chain oxidation pathway that degrades other vitamin D metabolites in bovine parathyroid cells. Both compounds compete for the same initial enzyme in the metabolic pathway, presumably the vitamin D-24 hydroxylase. We have shown that 1,25-$(OH)_2D_3$ induces its own degradation in NHPM through activation of the C-24 oxidation pathway (13). 1,25-$(OH)_2$-22oxa-$D_3$ has a potency similar to that of the parent hormone to stimulate 1,25-$(OH)_2D_3$ breakdown by macrophages. We have examined the ability of NHPM to metabolize 1,25-$(OH)_2$-22oxa-$D_3$ and whether the analog competes with 1,25-$(OH)_2D_3$ for the hydroxylases involved in 1,25-$(OH)_2D_3$ degradation. Time-course experiments showed that there was no detectable basal activity of the hydroxylases involved in 1,25-$(OH)_2$-22oxa-$D_3$ catabolism in NHPM. However, after 3-h exposure to 0.26 nM 1,25-$(OH)_2$-22oxa-$D_3$, we observed a significant induction of 1,25-$(OH)_2$-22oxa-$D_3$ degradation. This suggests that 1,25-$(OH)_2$-22oxa-$D_3$ induces its own catabolism through a genomic effect.

Our competition experiments suggested that 1,25-$(OH)_2$-22oxa-$D_3$ is less accessible than 1,25-$(OH)_2D_3$ for the enzyme(s) involved in vitamin D catabolism. A 10-fold molar excess of 1,25-$(OH)_2D_3$ totally blocked 1,25-$(OH)_2$-22oxa-$D_3$ catabolism. 1,25-$(OH)_2D_3$ catabolism was not affected by the presence of a 10-fold molar excess of radioinert 1,25-$(OH)_2$-22oxa-$D_3$; a 1000-fold excess of 1,25-$(OH)_2$-22oxa-$D_3$ was necessary to significantly reduce 1,25-$(OH)_2D_3$ degradation. $K_m$ determinations demonstrated that the apparent $K_m$ for 1,25-$(OH)_2$-22oxa-$D_3$ was higher than that for 1,25-$(OH)_2D_3$. Moreover, coincubation of 1,25-$(OH)_2$-22oxa-$D_3$ and 1,25-$(OH)_2D_3$ further increased the $K_m$ for 1,25-$(OH)_2$-22oxa-$D_3$. However, this 2-fold difference in the $K_m$s for 1,25-$(OH)_2D_3$ and 1,25-$(OH)_2$-22oxa-$D_3$ cannot explain the need for a 1000-fold excess of radioinert 1,25-$(OH)_2$-22oxa-$D_3$ to reduce 1,25-$(OH)_2D_3$ degradation by only 20%. It is apparent that other factors determine that when both metabolites are available for the cells, 1,25-$(OH)_2D_3$ will be preferentially degraded. A potential explanation is the existance of a delivery mechanism that markedly favors 1,25-$(OH)_2D_3$ access to the intracellular organelles responsible for vitamin D catabolism.

In summary, in normal peripheral monocytes, the hydroxylases involved in vitamin D catabolism have lower affinity for 1,25-$(OH)_2$-22oxa-$D_3$ than 1,25-$(OH)_2D_3$, with a resulting prolonged biological half-life of the analog. Clearly, the ability to degrade 1,25-$(OH)_2$-22oxa-$D_3$ and 1,25-$(OH)_2D_3$ differs between normal monocytes and parathyroid cells. Differential intracellular metabolism of 1,25-$(OH)_2$-22oxa-$D_3$

**TABLE 1.** Kinetic parameters of 1,25-$(OH)_2$-22oxa-$D_3$ and 1,25-$(OH)_2D_3$ catabolism by normal human mononuclear leukocytes

| | $K_m$ (nM) | $V_{max}$ (fmol/μg DNA·h) |
|---|---|---|
| 1,25-$(OH)_2D_3$ (n = 4) | 0.9 ± 0.2 | 37.0 ± 3.0 |
| 1,25-$(OH)_2$-22oxa-$D_3$ (n = 6) | 2.0 ± 0.8[a] | 58.2 ± 3.4[b] |
| 1,25-$(OH)_2$-22oxa-$D_3$ + 1,25-$(OH)_2D_3$ (n = 5) | 3.2 ± 0.8[c] | 59.3 ± 1.5 |

Normal human peripheral monocytes were incubated with increasing concentrations (0.13, 0.26, 0.52, and 0.78 nM) of tritiated 1,25-$(OH)_2$-22oxa-$D_3$ or 1,25-$(OH)_2D_3$. $K_m$ and $V_{max}$ were obtained using the double reciprocal plot of Lineweaver and Burk with linear regression analysis. The inhibitory effect of 1,25-$(OH)_2D_3$ on 1,25-$(OH)_2$-22oxa-$D_3$ catabolism was tested with the four concentrations (0.13, 0.26, 0.52, and 0.78 nM) of tritiated 1,25-$(OH)_2$-22oxa-$D_3$ and 0.65 nM radioinert 1,25-$(OH)_2D_3$. Data are expressed as the mean ± SEM. n, Number of independent experiments.

[a] Differs from 1,25-$(OH)_2D_3$, $P < 0.001$.

[b] Differs from 1,25-$(OH)_2D_3$, $P < 0.05$.

[c] Differs from 1,25-$(OH)_2$-22oxa-$D_3$, $P < 0.001$.

in different target tissues may be an additional factor involved in the selectivity of the analogs.

These results suggest a potential for 1,25-$(OH)_2$-22oxa-$D_3$ in the control of the hypercalcemia caused by deregulated calcitriol production in sarcoidosis and other granulomatoses. In peripheral monocytes, 1,25-$(OH)_2$-22oxa-$D_3$ can block 1,25-$(OH)_2D_3$ synthesis and induce 1,25-$(OH)_2D_3$ catabolism with the same potency of 1,25-$(OH)_2D_3$ (13). However, competition experiments showed that in the presence of a 1000-fold excess of the analog, 1,25-$(OH)_2D_3$ will be catabolized preferentially. As this analog was reported to have very weak calcemic action (17, 19, 21), 1,25-$(OH)_2$-22oxa-$D_3$ may provide an alternative therapeutic maneuver to reduce serum calcitriol and, therefore, serum calcium in sarcoidosis and other granulomatoses without the alterations in the immune system and other side-effects caused by the classical treatment with glucocorticoids.

## Acknowledgment

The authors thank Rhonda Coursey-Pratt for her assistance in the preparation of the manuscript.

## References

1. **Weissman J, Harall A, Eldestein S, Darid M, Spiren Z, Golander A** 1979 1-Alpha 25-dihydroxy vitamin $D_3$ and 24, 25-dihydroxyvitamin $D_3$ *in vitro* synthesis by human decidua and placenta. Nature 81:317–319
2. **Tanaka Y, Halloran B, Schnoes HK, DeLuca HF, Adams KG** 1979 Synthesis of 1,25-dihydroxyvitamin $D_3$ by rat placental tissue. Proc Natl Acad Sci USA 76:5033–5035
3. **Howard GA, Turner RT, Sherrard DJ, Baylink DJ** 1981 Human bone cells in culture metabolize 25-hydroxyvitamin $D_3$. J Biol Chem 256:7738–7740
4. **Bikle DD, Nemanic MK, Whitney JO, Elias PW** 1986 Neonatal human foreskin keratinocytes produce 1,25-dihydroxyvitamin $D_3$. Biochemistry 25:1545–1548
5. **Bikle DD, Nemanic MK, Gee E, Elias P** 1986 1,25-Dihydroxyvitamin $D_3$ production by human keratinocytes. Kinetics and regulation. J Clin Invest 78:557–566
6. **Adams JS, Sharma OR, Gacad MA, Singer FR** 1983 Metabolism of 25 hydroxy-vitamin $D_3$ by cultured pulmonary alveolar macrophages in sarcoidosis. J Clin Invest 78:1856–1860
7. **Cadranel J, Hance AJ, Milleron B, Paillard F, Akoun GM, Garabedian M** 1988 Vitamin $D_3$ metabolism in tuberculosis. Production of 1,25-$(OH)_2D_3$ by cells recovered by bronchioalveolar lavage and the role of this metabolite in calcium homeostasis. Am Rev Respir Dis 138:984–989
8. **Koeffler HP, Reichel H, Bishop JE, Norman AW** 1985 $\gamma$-Interferon stimulates production of 1,25-dihydroxyvitamin $D_3$ by normal human macrophages. Biochem Biophys Res Commun 127:596–600
9. **Reichel H, Koeffler HP, Bishop JE, Norman AW,** 1987 25-Hydroxyvitamin $D_3$ metabolism by lipopolysaccharide stimulated normal human macrophages. J Clin Endocrinol Metab 64:1–9
10. **Reichel H, Koeffler HP, Norman AW,** 1987 Synthesis *in vitro* of 1,25-dihydroxyvitamin $D_3$ interferon-$\gamma$ stimulated normal human bone marrow and alveolar macrophages. J Biol Chem 262:10931–10937
11. **Dusso AS, Finch J, Brown A, Ritter C, Delmez J, Schreiner G, Slatopolsky E** 1991 Extrarenal production of calcitriol in normal and uremic humans. J Clin Endocrinol Metab 72:157–164
12. **Dusso AS, Negrea L, Gunawardahama S, Lopez-Hilker S, Finch J, Mori T, Nishii Y, Slatopolsky E, Brown A** 1991 On the mechanism for the selective action of vitamin D analogs. Endocrinology 128:1687–1692
13. **Dusso A, Kamimura S, Gallieni M, Mori T, Nishii Y, Slatopolsky E** 1992 Modulation of vitamin D metabolism by 1,25-$(OH)_2D_3$ and 22-oxacalcitriol in normal human macrophages. J Bone Mineral Res 7:S151 (Abstract)
14. **Jones G, Vriezen D, Lohnes D, Palda V, Edwards NS** 1987 Side-chain hydroxylation of vitamin $D_3$ and its physiological implications. Steroids 49:29–53
15. **Reddy GS, Tseng K** 1989 Calcitroic acid, end product of renal metabolism of 1,25 dihydroxyvitamin $D_3$ though C-24 oxidation pathway 28:1763–1770
16. **Grieff M, Dusso A, Mori T, Nishii Y, Slatopolsky E, Brown AJ** 1992 22-Oxacalcitriol suppresses 25-hydroxycholecalciferol-1$\alpha$-hydroxylase in rat kidney. Biochem Biophys Res Commun 185:191–196
17. **Abe J, Morikawa M, Miyamoto K, Kaiho S, Fukushima M, Miyaura C, Abe E, Suda T, Nishii Y** 1987 Synthetic analogues of vitamin $D_3$ compounds with an oxygen atom in the side chain. FEBS Lett 226:58–62
18. **Binderup L, Bramm E** 1988 Effects of a novel vitamin D analogue MC903 on cell proliferation and differentiation *in vitro* and on calcium metabolism *in vivo*. Biochem Pharmacol 37:889–895
19. **Abe J, Takita Y, Nakano T, Miyaura C, Suda T, Nishii Y** 1989 A synthetic analog of vitamin $D_3$, 22-oxa-1$\alpha$,25-dihydroxyvitamin $D_3$, is a potent modulator of *in vivo* immunoregulating activity without inducing hypercalcemia in mice. Endocrinology 124:2645–2647
20. **Morimoto S, Imanaka S, Koh E, Shiraishi T, Nabata T, Kitano S, Miyashita Y, Nishii Y, Ogihara T** 1989 Comparisons of the inhibition of proliferation of normal and psoriatic fibroblasts by 1,25-dihydroxyvitamin $D_3$ and synthetic analogues of vitamin $D_3$ with an oxygen atom in their side chain. Biochem Int 19:1143–1149
21. **Takizawa M, Fallon M, Stein B, Epstein S** 1992 The effect of a new vitamin D analog, 22-oxa-1$\alpha$,25$(OH)_2D_3$, on bone mineral metabolism in normal male rats. Calcif Tissue Int 50:521–523
22. **Brown AJ, Ritter CR, Finch JL, Morrissey J, Martin KJ, Murayama E, Nishii Y, Slatopolsky E** 1989 The noncalcemic analogue of vitamin D, 22-oxacalcitriol, suppresses parathyroid hormone synthesis and secretion. J Clin Invest 84:728–732
23. **Okano T, Tsugawa N, Masuda S, Takeuchi A, Kobayashi T, Nishii Y** 1989 Protein-binding properties of 22-oxa-1$\alpha$,25-dihydroxyvitamin $D_3$, a synthetic analogue of 1$\alpha$,25-dihydroxyvitamin $D_3$. J Nutr Sci Vitaminol 35:529–533
24. **Reinhardt TA, Horst RL, Orf W, Hollis BW** 1984 A microassay for 1,25-dihydroxyvitamin $D_3$ not requiring high performance liquid chromatography: application to clinical studies. J Clin Endocrinol Metab 58:91–98
25. **Le Pecq JB, Paoletti C** 1966 A new fluorometric method for RNA and DNA determination. Anal Biochem 17:100–107
26. **Dusso AS, Negrea L, Finch J, Kamimura S, Lopez-Hilker S, Mori T, Nishii Y, Brown A, Slatopolsky E** 1992 The effect of 22-oxacalcitriol on serum calcitriol. Endocrinology 130:3129–3134
27. **Kumar R** 1986 Metabolism and mechanism of action of 1,25-dihydroxyvitamin $D_3$. Kidney Int 30:793–803
28. **Jones G, Vriezen D, Lohnes D, Palda V, Edwards NS** 1987 Side chain hydroxylation of vitamin $D_3$ and its physiological implications. Steroids 49:29–35
29. **Reddy GS, Tserng KY** 1989 Calcitroic acid, end product of renal metabolism of 1,25-dihydroxyvitamin $D_3$ through C-24 oxidation pathway. Biochemistry 28:1763–1769
30. **Brown AJ, Berkoben M, Ritter C, Mori T, Nishii Y, Slatopolsky E** 1992 Metabolism of 22-oxacalcitriol by a vitamin D-inducible pathway in cultured parathyroid cells. Biochem Biophys Res Commun 189:759–764