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Pharmacokinetics and Systemic Effect on Calcium Homeostasis of 1α,24-Dihydroxyvitamin D_2 in Rats

COMPARISON WITH 1α,25-DIHYDROXYVITAMIN D_2, CALCITRIOL, AND CALCIPOTRIOL

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ABSTRACT. 1α,24-Dihydroxyvitamin D_2 (1α,24$(OH)_2D_2$) is a metabolite of 1α-hydroxyvitamin D_2 (1α-OH-D_2), a prodrug in development as a treatment for secondary hyperparathyroidism occurring in end stage renal disease. This prodrug has a broader therapeutic index than the corresponding vitamin D_3 analogue, possibly because hepatic metabolism of 1α-OH-D_2 shifts at higher dose levels from 1α,25-dihydroxyvitamin D_2 (1α,25$(OH)_2D_2$) to 1α,24$(OH)_2D_2$. In this report, we present the pharmacokinetics of 1α,24$(OH)_2D_2$ and its systemic effects on serum and urine calcium in rats. These properties were compared with those of 1α,25$(OH)_2D_2$, calcitriol, the active metabolite of endogenous vitamin D_3, and calcipotriol, a vitamin D analogue noted for its rapid clearance and minimal effect on calcium homeostasis. Comparison of the blood concentration curves from time zero to infinity indicated that 1α,24$(OH)_2D_2$ had about one-fifth the systemic exposure of 1α,25$(OH)_2D_2$ or calcitriol, but almost 30 times that of calcipotriol. The oral bioavailabilities and circulating half-lives of 1α,24$(OH)_2D_2$ and calcitriol were similar, whereas those of calcipotriol were much less. In vitamin D-deficient rats, oral doses of 1α,25$(OH)_2D_2$ and calcitriol produced similar dose-dependent increases in serum calcium, whereas an oral dose 30 times greater was required for 1α,24$(OH)_2D_2$ to produce a similar response. Dose–response curves generated after oral and subcutaneous administration of 1α,24$(OH)_2D_2$, calcitriol, and calcipotriol to normal rats indicated that 1α,24$(OH)_2D_2$ increases serum and urine calcium to a much lesser extent than calcitriol, and to a slightly greater extent than calcipotriol. These properties of 1α,24$(OH)_2D_2$ suggest that production of this metabolite from 1α-OH-D_2 contributes to the lowered toxicity of 1α-OH-D_2 and indicate that 1α,24$(OH)_2D_2$ itself has therapeutic potential. BIOCHEM PHARMACOL 53;6:829–837, 1997.

KEY WORDS. vitamin D analogs; 1α,24-dihydroxyvitamin D_2; calcipotriol; 1α,25-dihydroxyvitamin D_3; 1α,25-dihydroxyvitamin D_2; pharmacokinetics; calcium

Vitamin D_3 must be metabolized to its dihydroxylated, hormonal form, 1α,25-dihydroxyvitamin D_3 (calcitriol), before it will bind to its intracellular VDR† with high affinity [1]. Its binding to the VDR initiates events resulting in regulation of calcium homeostasis, namely control of intestinal absorption of dietary calcium, the conservation of calcium by the kidney, and the mobilization of calcium from the skeleton. Collectively, these functions are termed the calcemic activity of the vitamin. Binding of calcitriol to the VDR also has a role in the regulation of cell proliferation and differentiation [2].

The use of vitamin D metabolites and analogs to treat hyperproliferative disorders has been limited by their calcemic activity. New vitamin D compounds, therefore, have been sought which exert antiproliferative activities with minimal unwanted effects on serum calcium. Often, potential candidates have alterations either in the binding to the VDR and to the serum DBP, and/or in their intracellular metabolism [3].

Vitamin D_2, in contrast to vitamin D_3, can be metabolized *in vivo* to 24-hydroxyvitamin D_2 as well as 25-hydroxyvitamin D_2 [4] and subsequently to the dihydroxylated forms of 1α,24$(OH)_2D_2$ and 1α,25$(OH)_2D_2$ [5]. This alternate route of metabolism to the 24-hydroxylated compounds has been observed when large doses of vitamin D_2 have been administered to pigs, rats, and cows. Recently, Mawer *et al.*‡ and Davies and colleagues [6] have reported

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† *Abbreviations:* VDR, vitamin D receptor; 1α,24$(OH)_2D_2$, 1α,24(S)-dihydroxyvitamin D_2; calcitriol, 1α,25-dihydroxyvitamin D_3; DBP, vitamin D binding protein; 1α-OH-D_2, 1α-hydroxyvitamin D_2; 1α-OH-D_3, 1α-hydroxyvitamin D_3; 1α,25$(OH)_2D_2$, 1α,25-dihydroxyvitamin D_2; C_{max}, maximum blood concentration; $T_{1/2}$, half-life; and $AUC_{0-\infty}$, area under the curve from 0 hr to infinity.

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‡ Mawer EB, Davies M, Still PE, Jones G, Knutson JC and Bishop CW, 1,24(S)-Dihydroxyvitamin D_2, a biologically active analogue of vitamin D_2, is a naturally occurring metabolite in humans. Abstract, Bone and Tooth Society Meeting, 1995, Warwick, U.K.

$1\alpha,24(OH)_2D_2$ in the blood of vitamin D-deficient patients administered pharmacologic doses of vitamin D_2.

Similarly, the vitamin D_2 analog 1α-OH-D_2 can be metabolized to $1\alpha,24(OH)_2D_2$ and $1\alpha,25(OH)_2D_2$. Study of 1α-OH-D_2 metabolism in cultured human hepatoma cells showed that the production of $1\alpha,24(OH)_2D_2$ increases and that of $1\alpha,25(OH)_2D_2$ decreases as the concentration of the 1α-OH-D_2 substrate is raised. In contrast, only traces of a $1\alpha,24$-dihydroxylated metabolite are produced when 1α-OH-D_3 is used as the substrate, even at high concentrations [7]. Study of 1α-OH-D_2 metabolism *in vivo* showed a similar shift from $1\alpha,25(OH)_2D_2$ to $1\alpha,24(OH)_2D_2$ as doses increased [8]. The $1\alpha,24$-dihydroxylated metabolite binds to the VDR with high affinity (greater than half that of calcitriol) and to the DBP with one-tenth the affinity of calcitriol [7]. $1\alpha,24(OH)_2D_2$ is as active as, or slightly less active than, calcitriol in growth hormone and chloramphenicol acetyltransferase reporter gene expression systems [7] and in inhibiting the growth of cultured human keratinocytes [9]. Thus, $1\alpha,24(OH)_2D_2$ has potent biological activity.

Numerous animal studies [10–14] have demonstrated that 1α-OH-D_2 is less toxic than 1α-OH-D_3 in terms of LD_{50}, nephrocalcinosis, hypercalciuria, and hypercalcemia, despite equivalent activity on intestinal calcium transport and bone mobilization. To determine whether the production of the alternate metabolite, $1\alpha,24(OH)_2D_2$, contributes to the lower toxicity of 1α-OH-D_2, we investigated the calcemic effects of systemically administered $1\alpha,24(OH)_2D_2$ as well as its pharmacokinetics. We compared these properties of $1\alpha,24(OH)_2D_2$ with those of the most well-known active metabolite of vitamin D_2, $1\alpha,25(OH)_2D_2$, and two active vitamin D_3 compounds noted for their different pharmacokinetics and effects on calcium homeostasis, namely calcitriol and calcipotriol. Calcitriol and $1\alpha,25(OH)_2D_2$ possess potent calcemic activity and circulate readily. Calcipotriol, a vitamin D_3 analogue used topically for the treatment of psoriasis, has a high clearance rate from the blood [15, 16] and, therefore, a low calcemic activity [17].

Our findings indicate that $1\alpha,24(OH)_2D_2$ increases systemic calcium to a much lesser extent than either $1\alpha,25(OH)_2D_2$ or calcitriol, but to a slightly greater extent than calcipotriol, while the pharmacokinetics of $1\alpha,24(OH)_2D_2$ approximate those of $1\alpha,25(OH)_2D_2$ and calcitriol, but not calcipotriol. Together, these properties of $1\alpha,24(OH)_2D_2$ suggest that production of $1\alpha,24(OH)_2D_2$ contributes to the lowered toxicity of 1α-OH-D_2. The potent biological activity of $1\alpha,24(OH)_2D_2$ in combination with lowered calcemic activity suggests that $1\alpha,24(OH)_2D_2$ itself has therapeutic potential. Parts of this work have been published previously in a preliminary form [18].*

MATERIALS AND METHODS

Vitamin D Compounds and Assay Reagents

$1\alpha,24(OH)_2D_2$ was synthesized as previously described [7]. $1\alpha,25(OH)_2D_2$ was synthesized by Steroids, Ltd. (Chicago, IL); calcitriol and calcipotriol were synthesized by Albany Molecular Research (Albany, NY).

Components for the radioreceptor assays, [26,27-^{3}H]-$1\alpha,25(OH)_2D_3$ and calf thymus receptor, were purchased from Incstar (Stillwater, MN).

Rats

Rats were purchased from Holtzman Lab Animals (Madison, WI) or Charles River Laboratories (Wilmington, MA).

Blood Assays for Vitamin D Compounds

Assay methods for $1\alpha,24(OH)_2D_2$, $1\alpha,25(OH)_2D_2$, and calcitriol have been reported previously [8], while that for calcipotriol has been submitted for publication.†

Briefly, each plasma or serum sample (0.25 to 1.0 mL, or total available sample) was extracted with acetonitrile, and the acetonitrile extract was fractionated using a C_{18}OH solid-phase extraction cartridge [19] followed by normal-phase HPLC. After HPLC separation, each compound of interest was quantified using a non-equilibrium competitive radioreceptor assay based on the bovine thymus VDR. Concentrations of $1\alpha,24(OH)_2D_2$ and calcipotriol were determined using a $1\alpha,24(OH)_2D_2$ or calcipotriol standard curve, respectively, while $1\alpha,25(OH)_2D_2$ and calcitriol both were quantified from calcitriol standard curves. The standard curve ranges were 2 to 30 pg/tube for $1\alpha,24(OH)_2D_2$, and 1 to 25 pg/tube for $1\alpha,25(OH)_2D_2$, calcitriol, and calcipotriol. After allowance for typical sample volumes (0.75 mL), method recoveries (60%) and dilution factors, these standard curves corresponded to assay working ranges of approximately 5 to 67 pg/mL for $1\alpha,24(OH)_2D_2$ and 2.5 to 55 pg/mL for the other compounds. A "less than" sign was placed before mean values that included at least one determination lower than the limit of detection. The arithmetic mean in these cases was obtained by assigning a numerical value to the limit of detection. The limit of detection (typically 2–10 pg/mL) is dependent on the volume of sample extracted and the volume assayed.

Clinical Chemistry Assays

Serum calcium and phosphate (expressed as mg phosphorus), and urine calcium and creatinine were analyzed with a Boehringer Mannheim Hitachi 704 analyzer. All reagents and controls for the colorimetric determinations were pur-

* LeVan LW, Knutson JC, Valliere CR and Bishop CW, Low-dose pharmacokinetics of calcipotriol in rats after oral and intravenous administration. Vitamin D: Actions and applications in dermatology. European Society of Dermatological Research: Clinically Oriented Symposium, p. 24, 1995.

† LeVan LW, Knutson JC, Valliere CR and Bishop CW, Manuscript submitted for publication.

chased from Boehringer Mannheim (Indianapolis, IN). For urine calcium determinations, the sample was acidified to pH 2 with HCl; for urine creatinine, the sample was diluted with saline or distilled water. Evaluation of equality of means was made by the one-way analysis of variance using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine significant differences from control means.

Studies in Vitamin D-Deficient Rats

Male weanling rats (3 weeks of age) were housed under incandescent lighting and fed a diet deficient in vitamin D with 0.47% calcium and 0.3% phosphorus (Diet TD89123, Teklad Premier Laboratory Diets, Madison, WI). After 3 weeks, the mean serum calcium of three representative rats was 6.3 mg/dL, indicating a deficiency in vitamin D. Groups of 10–12 rats were administered either $1\alpha,24(OH)_2D_2$, $1\alpha,25(OH)_2D_2$, or calcitriol orally in fractionated coconut oil at doses of 0.042, 0.25, and 1.5 μg/kg/day for 14 days. A control group received fractionated coconut oil only. All rats were killed 24 hr after the last dose, and the serum levels of calcium, phosphorus, and vitamin D compounds were determined.

Pharmacokinetic Studies in Normal Rats

The pharmacokinetics of $1\alpha,24(OH)_2D_2$, $1\alpha,25(OH)_2D_2$, and calcitriol following single oral doses were evaluated in 5- to 7-week-old female rats. Groups of five rats each were administered the compounds at 0.15 and 0.39 μg/kg in fractionated coconut oil. Animals were killed 1, 2, 3, 4, 6, 9, 15, and 24 hr after dosing. Plasma was collected and subsequently analyzed for the appropriate vitamin D compound. Control rats, who received only the vehicle, were killed at 0, 3, and 15 hr post-dose.

The pharmacokinetics and bioavailability of $1\alpha,24(OH)_2D_2$ and calcipotriol following single oral or intravenous doses were evaluated in 7- to 9-week-old female rats. Compounds were administered (0.39 μg/kg) orally, in fractionated coconut oil, or intravenously, in ethanol. Groups of five rats were exsanguinated 5, 10, 20, and 30 min and 1, 2, 4, and 24 hr post-dosing. A group of rats exsanguinated prior to dosing served as the control group. Plasma was collected and subsequently analyzed for the appropriate vitamin D compound.

The C_{max} values were recorded from observed data. The circulating $T_{1/2}$ values were obtained by linear regression of the terminal phase of log-linear concentration-time profiles. $AUC_{0-\infty}$ was estimated by linear trapezoidal approximation with end correction to infinite time.

Studies of Calcium Homeostasis in Normal Rats

ORAL ADMINISTRATION. Male rats, 7 weeks of age, were housed in metabolism cages for 7 days. Groups of five rats received $1\alpha,24(OH)_2D_2$ calcipotriol, or vehicle (fractionated coconut oil) daily as an oral dose. Animals were dosed with $1\alpha,24(OH)_2D_2$ or calcipotriol at 3, 10, 30, and 100 μg/kg/day. In a continuation study, additional rats were dosed orally with calcitriol at 0.1, 0.3, 1.0, and 3.0 μg/kg/day. Five consecutive 24-hr urine collections were obtained from all rats beginning immediately after the third dose and ending at the time of death, 24 hr after the last dose. At this time, blood was collected for serum calcium and phosphorus determinations and vitamin D compound analysis. Clinical chemistry data are expressed as the ratio of mean 5-day urine calcium concentration to mean 5-day urine creatinine concentration.

SUBCUTANEOUS ADMINISTRATION. Single subcutaneous injections of $1\alpha,24(OH)_2D_2$, calcipotriol, calcitriol, or vehicle (ethanol) were administered to 7 to 9-week-old male rats. Groups of five rats each were dosed with $1\alpha,24(OH)_2D_2$ or calcipotriol at 0.3, 1.0, 3.0, 10, or 100 μg/kg. Rats receiving calcitriol were dosed with 0.01, 0.03, 0.1, 0.3, 1.0, 10, and 100 μg/kg. The rats voided their bladders upon injection and were then placed in metabolism cages for 24-hr urine collections. Rats were killed 24 hr post-dose, and serum was prepared and analyzed for calcium, phosphorus, and vitamin D compounds. Urine was analyzed for calcium and creatinine.

In a separate study, male rats 7–9 weeks of age were injected subcutaneously daily for 3 consecutive days with 3 μg/kg of $1\alpha,24(OH)_2D_2$, calcipotriol, calcitriol, or the ethanol vehicle and placed in metabolism cages. Three 24-hr urine collections were obtained, each started immediately after dosing. All animals were exsanguinated 24 hr after the third dosing, and serum was prepared and analyzed for calcium, phosphorus, and vitamin D compounds. Urine was analyzed for calcium and creatinine.

RESULTS

Studies in Vitamin D-Deficient Rats

Serum calcium increased in vitamin D-deficient rats administered $1\alpha,24(OH)_2D_2$ orally for 14 days. As shown in Table 1, $1\alpha,24(OH)_2D_2$ elicited nominal dose-related increases in serum calcium which reached statistical significance only at the highest dosage. In contrast, $1\alpha,25(OH)_2D_2$ and calcitriol produced large dose-related increases in serum calcium, all of which were statistically significant. Serum phosphorus was increased significantly in all groups administered $1\alpha,24(OH)_2D_2$, in the groups receiving calcitriol at the two lower dosages, and the group receiving $1\alpha,25(OH)_2D_2$ at the lowest dosage.

Pharmacokinetic Studies in Normal Rats

Three compounds, namely $1\alpha,24(OH)_2D_2$, $1\alpha,25(OH)_2D_2$, and calcitriol, were administered to normal rats in single oral doses (0.39 μg/kg), and the serum concentrations of these compounds determined (Fig. 1). As shown in Table 2,

TABLE 1. Effects of 1α,24(OH)$_2$D$_2$, 1α,25(OH)$_2$D$_2$, and calcitriol on serum calcium and phosphorus in vitamin D-deficient rats

Compound	Dose (μg/kg/day)	Serum calcium (mg/dL)	Serum phosphorus (mg/dL)
Vehicle	0	5.4 ± 0.28 (12)	11.5 ± 1.05 (12)
1α,24(OH)$_2$D$_2$	0.042	5.9 ± 0.61 (12)	13.5 ± 1.35* (12)
	0.250	6.0 ± 0.75 (10)	13.6 ± 1.07* (10)
	1.500	7.4 ± 1.51* (11)	13.7 ± 1.21* (11)
1α,25(OH)$_2$D$_2$	0.042	7.8 ± 1.66* (9)	14.0 ± 2.37* (9)
	0.250	8.8 ± 1.70* (9)	12.9 ± 1.63 (9)
	1.500	11.2 ± 0.86* (10)	11.9 ± 0.99 (10)
Calcitriol	0.042	8.1 ± 1.15* (8)	14.7 ± 1.61* (8)
	0.250	10.1 ± 1.84* (10)	13.9 ± 1.73* (10)
	1.500	12.3 ± 1.19* (10)	13.2 ± 1.40 (10)

Vitamin D compounds were administered orally, once daily for 14 days to vitamin D-deficient rats. Values are means ± SD. The number of animals/group appears in parenthesis.

* $P < 0.01$ vs vehicle control.

the C_{max} of 1α,24(OH)$_2$D$_2$ in the serum was considerably less than that of 1α,25(OH)$_2$D$_2$ or calcitriol; however, the $T_{1/2}$ values of all three compounds in the blood were similar, being approximately 5 hr. The time at which the maximum concentration occurred (t_{max}) for all three compounds was 1 hr, the first time sampled.

TABLE 2. Pharmacokinetic parameters for 1α,24(OH)$_2$D$_2$, 1α,25(OH)$_2$D$_2$, and calcitriol after single oral doses (0.39 μg/kg)

Compound	Baseline (pg/mL)	C_{max} (pg/mL)	$AUC_{0-\infty}$ ((pg · hr)/mL)	$T_{1/2}$ (hr)
1α,24(OH)$_2$D$_2$	ND*	89.4	659	4.9
1α,25(OH)$_2$D$_2$	ND	363.9	2676	5.1
Calcitriol	67.0	491.6	3690	5.8

* ND = not detectable (<10 pg/mL).

In a pharmacokinetics/bioavailability study, 1α,24(OH)$_2$D$_2$ and calcipotriol (0.39 μg/kg) were administered intravenously, as well as orally. Following oral administration, plasma concentrations of 1α,24(OH)$_2$D$_2$ increased until 2 hr post-dose (C_{max} = 140.3 pg/mL) and then decreased in an approximately monoexponential manner (Figs. 1 and 2). Following intravenous administration, the plasma concentration of 1α,24(OH)$_2$D$_2$ (C_{max} = 2201 pg/mL) decreased in a biexponential manner (Fig. 2). Terminal elimination rate constants and values for $T_{1/2}$ were similar for both routes of administration. Absolute oral bioavailability of 1α,24(OH)$_2$D$_2$ based on $AUC_{0-\infty}$ (p.o.) and $AUC_{0-\infty}$ (i.v.) was 16.2%. This bioavailability was very similar to that observed for 1α,25(OH)$_2$D$_3$ (~15%) in a parallel experiment (data not shown).

Comparison of blood levels of 1α,24(OH)$_2$D$_2$ and calcipotriol after single oral doses of 0.39 μg/kg highlight the large difference in systemic availability of these two compounds (Fig. 3). After oral administration of calcipotriol, the C_{max} (28 pg/mL) was observed at 10 min post-dose, and the plasma concentration had decreased to an undetectable level by 1 hr. The values for $T_{1/2}$ for calcipotriol were 0.6 and 0.9 hr for oral and intravenous dosing, respectively.

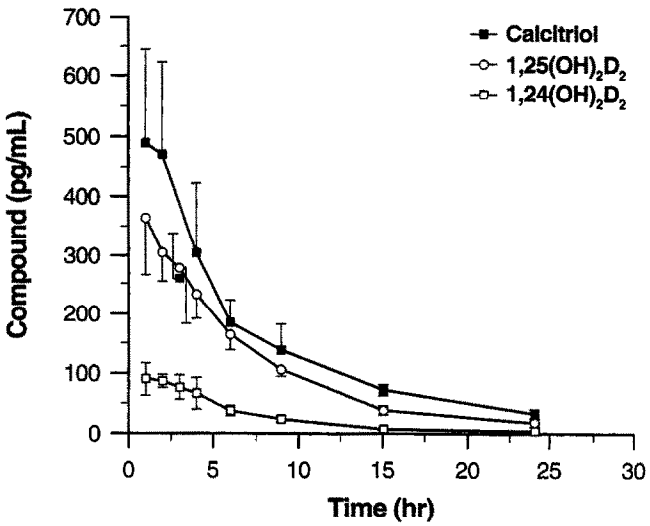

FIG. 1. Plasma levels of calcitriol, 1α,25(OH)$_2$D$_2$, and 1α,24(OH)$_2$D$_2$ following single oral doses (0.39 μg/kg) to normal rats. Each value represents the mean ± SD of five rats.

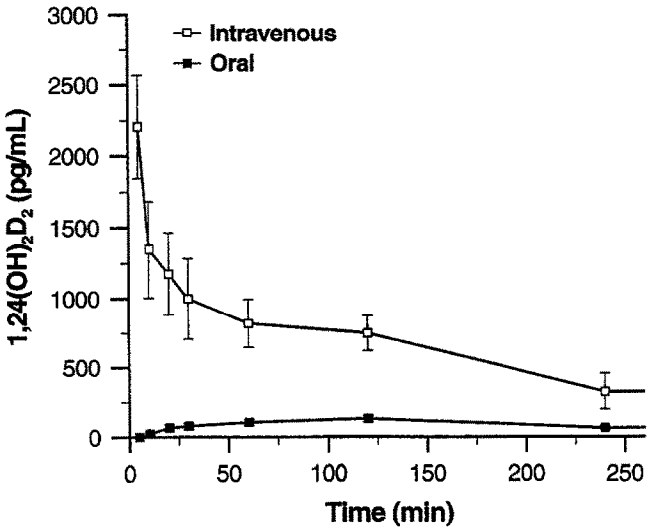

FIG. 2. Plasma levels of 1α,24(OH)$_2$D$_2$ following single doses of 1α,24(OH)$_2$D$_2$ (0.39 μg/kg) administered intravenously and orally to normal rats. Each value represents the mean ± SD of five rats.

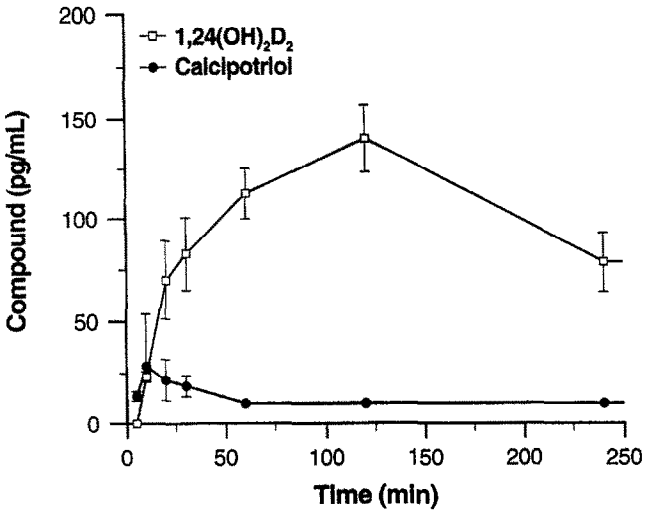

FIG. 3. Plasma levels of $1\alpha,24(OH)_2D_2$ and calcipotriol following single oral doses (0.39 μg/kg) to normal rats. Each value represents the mean ± SD of five rats.

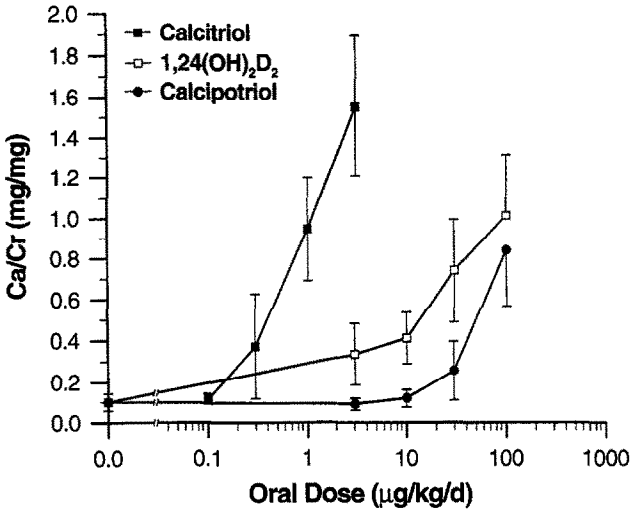

FIG. 4. Effects of calcitriol, $1\alpha,24(OH)_2D_2$, and calcipotriol on urinary calcium excretion after seven daily oral doses to normal rats. Calcium and creatinine were determined in 24-hr urine samples and expressed as the ratio of the means of calcium to creatinine from day 3 to day 7. Values represent means ± SD for five rats.

The $AUC_{0-\infty}$ for $1\alpha,24(OH)_2D_2$ was 683 (pg · hr)/mL, while that of calcipotriol was 25.3 (pg · hr)/mL. Unlike $1\alpha,24(OH)_2D_2$ and calcitriol, calcipotriol had a bioavailability of less than 10%. Based on a comparison of AUC_{0-t}, which avoids the large percentage estimate for $AUC_{t-\infty}$ for this compound, the bioavailability of calcipotriol was about 5%.

Studies of Calcium Homeostasis in Normal Rats

The dose–response increases in urinary calcium produced by $1\alpha,24(OH)_2D_2$, calcipotriol, and calcitriol were compared in normal rats after 7 consecutive daily oral doses. The results, shown in Figure 4, indicate that calcitriol was approximately 30 and 80 times more potent than $1\alpha,24(OH)_2D_2$ and calcipotriol, respectively, in causing increases in the urine calcium/creatinine ratio. Serum calcium was increased significantly ($P < 0.01$) from 11.1 ± 0.19 mg/dL (mean ± SD) to 12.3 ± 0.74 and 12.7 ± 0.61 with $1\alpha,24(OH)_2D_2$ doses of 30 and 100 μg/kg/day, respectively, but not at doses below 30 μg/kg/day. No significant change was observed in serum calcium with any dose level of calcipotriol. Calcitriol at the highest dose tested (3 μg/kg/day) produced a serum calcium of 12.7 ± 0.61 mg/dL. Serum phosphorus was not changed significantly by any of the three compounds at the dose levels tested.

The effects of $1\alpha,24(OH)_2D_2$, calcipotriol, and calcitriol on calcium excretion following single subcutaneous doses also were compared in normal rats. The results (Fig. 5) again show that calcitriol is more potent in causing hypercalcuria. Serum calcium levels were increased significantly ($P < 0.01$) for $1\alpha,24(OH)_2D_2$ and calcipotriol only at the highest dose (100 μg/kg) where values increased from 12.4 ± 0.4 mg/dL (mean ± SD) for the control to 15.0 ± 0.83 and 14.7 ± 0.42 mg/dL for $1\alpha,24(OH)_2D_2$ and calcipotriol, respectively. The serum calcium levels at dosages of 0.3, 1.0, 3.0, and 10 μg/kg were 12.1 ± 0.40, 12.4 ± 0.69, 12.4 ± 0.29, and 13.2 ± 0.36 mg/dL, respectively, for $1\alpha,24(OH)_2D_2$ and 12.2 ± 0.19, 12.1 ± 0.60, 12.3 ± 0.45, and 13.1 ± 1.09 mg/dL, respectively, for calcipotriol. Calcitriol at 1, 10, and 100 μg/kg significantly increased serum calcium ($P < 0.01$) to 14.4 ± 0.35, 15.1 ± 1.03, and 16.4 ± 0.92 mg/dL, respectively. Serum phosphorus increased significantly ($P < 0.01$) from a control value of 13.3 ± 1.39 mg/dL (mean ± SD) to 16.6 ± 0.9 with 100 μg/kg calcipotriol and to 17.2 ± 1.28, 17.1 ± 0.96, and 16.1 ± 1.14 ($P < 0.05$) with 1, 10, and 100 μg/kg calcitriol, respectively. $1\alpha,24(OH)_2D_2$ did not increase serum phosphorus significantly at the dose levels tested.

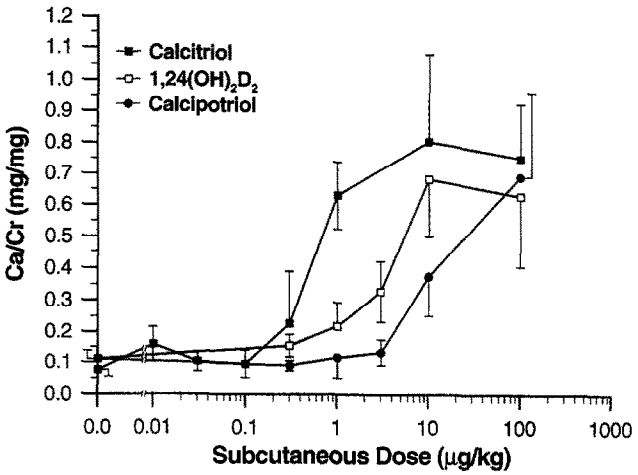

FIG. 5. Effects of calcitriol, $1\alpha,24(OH)_2D_2$, and calcipotriol on urinary calcium excretion after single subcutaneous doses to normal rats. Calcium and creatinine were determined in 24-hr urine samples and expressed as the ratio of calcium to creatinine. Values represent means ± SD for five rats.

TABLE 3. Serum levels of vitamin D compounds 24 hr after the last of fourteen daily oral doses to vitamin D-deficient rats

Compound administered	Dose (μg/kg/day)	Serum 1α,24(OH)$_2$D$_2$ (pg/mL)	Serum calcitriol (pg/mL)
Vehicle	0	<5.7 (12)	<10 (12)
1α,24(OH)$_2$D$_2$	0.042	<6.6 (12)	<10 (12)
	0.250	9.6 ± 2.8 (10)	<10 (10)
	1.500	16.2 ± 4.5 (11)	<10 (11)
Calcitriol	0.042	NA*	124.7 ± 25 (8)
	0.250	NA	121.8 ± 58 (10)
	1.500	NA	124.0 ± 72 (10)

Vitamin D compounds were administered orally, once daily for 14 days to vitamin D-deficient rats. Serum was prepared from blood obtained 24 hr after the last dose. The serum was extracted, the extract was separated by HPLC, and the fraction containing the vitamin D compound was quantified by radioreceptor assay using the bovine thymus vitamin D receptor. All values represent means; where error values are denoted, values are means ± SD. The number of animals/group appears in parentheses.

*NA = not applicable.

Subcutaneous administration of 1α,24(OH)$_2$D$_2$, calcipotriol, and calcitriol at 3 μg/kg/day for 3 consecutive days produced mean urine calcium/creatinine ratios (mean ± SD) of 0.548 ± 0.527, 0.149 ± 0.034, and 1.065 ± 0.431, respectively, relative to 0.113 ± 0.017 for a vehicle control. Serum calcium increased significantly ($P < 0.01$) from 12.3 ± 0.36 mg/dL (control) to 14.2 ± 0.77 and 16.7 ± 0.49 mg/dL with 1α,24(OH)$_2$D$_2$ and calcitriol, respectively. The serum calcium values after dosing with 1α,24(OH)$_2$D$_2$ and calcipotriol (11.9 ± 0.20 mg/dL) were significantly ($P < 0.01$) lower than after calcitriol.

Blood Levels of Vitamin D Compounds

Serum levels of 1α,24(OH)$_2$D$_2$ and calcitriol were measured 24 hr after the last of fourteen oral doses to the vitamin D-deficient rats, as shown in Table 3. Rats dosed with 1α,24(OH)$_2$D$_2$ showed a dose-related increase in the circulating levels of this compound, whereas circulating levels of calcitriol appeared similar at all dose levels. As expected from the original vitamin D-deficient state of the animals, the calcitriol levels in those animals dosed with 1α,24(OH)$_2$D$_2$ were low.

Repeated oral dosing of these compounds to normal rats produced the serum levels shown in Table 4. Twenty-four hours after the seventh dose, low or undetectable amounts of 1α,24(OH)$_2$D$_2$ or calcipotriol remained circulating; however, systemic effects of these compounds were apparent not only from changes in calcium homeostasis, but also from the suppression of the level of endogenous calcitriol. Essentially no calcitriol was detectable in the groups dosed with 1α,24(OH)$_2$D$_2$ or calcipotriol at 30 or 100 μg/kg/day. Surprisingly, calcipotriol, at a dose of 10 μg/kg/day, markedly reduced the level of calcitriol despite only a slight effect on urine or serum calcium.

TABLE 4. Serum levels of vitamin D compounds 24 hr after the last of seven daily oral doses to normal rats

Compound administered	Dose (μg/kg/day)	1α,24(OH)$_2$D$_2$ (pg/mL)	Calcipotriol (pg/mL)	Endogenous calcitriol (pg/mL)
Experiment 1				
Vehicle	0	<12.0	<6.3	88.4 ± 9.7
1α,24(OH)$_2$D$_2$	3	11.7 ± 4.2	NA*	45.9 ± 15.3
	10	<6.1	NA	<10.0
	30	<9.5	NA	<10.0
	100	7.7 ± 2.0	NA	<10.0
Calcipotriol	3	NA	<5.4	89.1 ± 8.8
	10	NA	<5.0	36.6 ± 17.2
	30	NA	<5.0	<10.0
	100	NA	<5.0	<24.8
Experiment 2				
Vehicle	0	NA	NA	85.1 ± 20.8
Calcitriol	0.1	NA	NA	65.4 ± 8.9
	0.3	NA	NA	40.4 ± 9.5
	1.0	NA	NA	50.6 ± 9.5
	3.0	NA	NA	64.5 ± 16.4

Vitamin D compounds were administered orally, once daily for 7 days to normal rats. Serum was prepared from blood obtained 24 hr after the last dose. The serum was extracted, the extract was separated by HPLC, and the fraction containing the vitamin D compound was quantified by radioreceptor assay using the bovine thymus vitamin D receptor. All values are means; where error values are denoted, values are means ± SD. All groups contained 5 rats.

*NA = not applicable.

TABLE 5. Serum levels of vitamin D compounds 24 hr after a single subcutaneous dose to normal rats

Test compound	Dose (μg/kg)	$1\alpha,24(OH)_2D_2$ (pg/mL)	Calcipotriol (pg/mL)	Calcitriol (pg/mL)
Experiment 1				
Vehicle	0	9.9 ± 2.6	5.6 ± 2.6	96.6 ± 21.6
$1\alpha,24(OH)_2D_2$	1	27.7 ± 11.6	NA*	33.9 ± 4.5
	10	167.4 ± 85.2	NA	9.2 ± 1.7
	100	1753.2 ± 474.5	NA	<7.4
Calcipotriol	1	NA	<6.6	65.1 ± 12.9
	10	NA	6.7 ± 1.0	20.4 ± 5.1
	100	NA	131.4 ± 59.8	<7.7
Calcitriol	1	NA	NA	444.0 ± 196.8
	10	NA	NA	1494.9 ± 348.4
	100	NA	NA	5338.6 ± 2812.6
Experiment 2				
Vehicle	0	12.8 ± 5.4	5.5 ± 1.3	78.0 ± 10.7
$1\alpha,24(OH)_2D_2$	0.3	23.3 ± 8.5	NA	48.0 ± 12.1
	3.0	59.4 ± 15.3	NA	18.8 ± 3.4
Calcipotriol	0.3	NA	9.7 ± 1.8	78.7 ± 11.0
	3.0	NA	5.6 ± 2.0	38.7 ± 4.8
Calcitriol	0.01	NA	NA	115.3 ± 26.1
	0.03	NA	NA	125.2 ± 15.6
	0.10	NA	NA	173.9 ± 16.0
	0.30	NA	NA	328.0 ± 67.0

Normal rats received a single subcutaneous dose of the vitamin D compound. Serum was prepared from blood obtained 24 hr later. The serum was extracted, the extract was separated by HPLC, and the fraction containing the vitamin D compound was quantified by radioreceptor assay using the bovine thymus vitamin D receptor. All values are means; where errors are denoted, values are means ± SD. All groups contained five rats.

*NA = not applicable.

A single subcutaneous dose of calcitriol greatly increased serum calcitriol levels relative to control levels (Table 5). Serum calcitriol decreased after $1\alpha,24(OH)_2D_2$ or calcipotriol administration (Fig. 6), even when the serum levels of the administered compounds were below the endogenous control level of calcitriol. Similar effects on serum levels were found after repeated subcutaneous doses (3 μg/kg) of these compounds (data not shown).

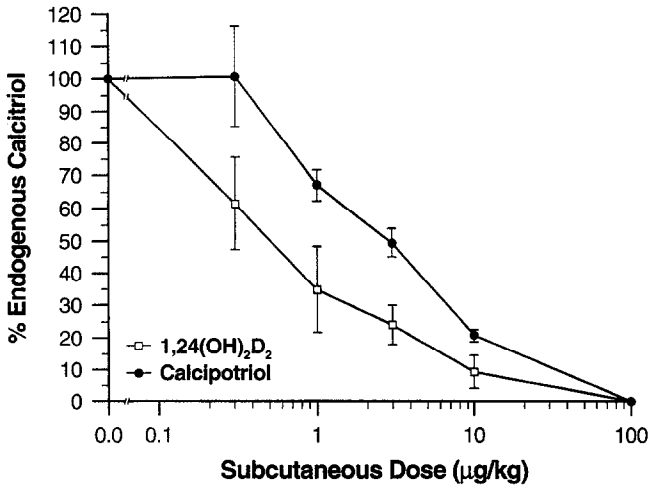

FIG. 6. Effects of $1\alpha,24(OH)_2D_2$ and calcipotriol on the serum levels of endogenous calcitriol 24 hr after a single subcutaneous dose. The data are expressed as a percentage of the endogenous calcitriol from control rats. (See experiments 1 and 2 of Table 5 for absolute values for control (vehicle) rats.) Each value represents the mean ± SD of five rats.

DISCUSSION

The current data show that systemic administration of $1\alpha,24(OH)_2D_2$, a metabolite of both vitamin D_2 and 1α-OH-D_2, affects calcium homeostasis to a much lesser extent than either $1\alpha,25(OH)_2D_2$ or calcitriol, but to a somewhat greater extent than calcipotriol. The relative calcemic activities of these compounds were evaluated by increases in serum calcium in vitamin D-deficient and normal rats, and by increases in calcium excretion in normal rats. Systemic exposure, as defined by $AUC_{0-\infty}$ following a single dose, was greatest for calcitriol, followed by $1\alpha,25(OH)_2D_2$, $1\alpha,24(OH)_2D_2$, and calcipotriol. The oral bioavailability and circulating $T_{1/2}$ of $1\alpha,24(OH)_2D_2$ approximated those of calcitriol, with those of calcipotriol being much less. By one biologic measure of systemic exposure, the reduction of serum levels of endogenous calcitriol, $1\alpha,24(OH)_2D_2$ was more potent than calcipotriol.

In comparing $1\alpha,24(OH)_2D_2$ to $1\alpha,25(OH)_2D_2$, we contrast the calcemic properties of the two known active metabolites formed after pharmacologic or physiologic doses of

vitamin D_2* [5] and 1α-OH-D_2 [7, 8]. 1α,25$(OH)_2D_2$ readily increased serum calcium in vitamin D-deficient rats, to the same extent as calcitriol. However, 1α,24$(OH)_2D_2$ required more than 35 times the dose of 1α,25$(OH)_2D_2$ (or calcitriol) to produce a similar increase in serum calcium.

In comparing 1α,24$(OH)_2D_2$ to calcipotriol and calcitriol, we characterized the properties of 1α,24$(OH)_2D_2$ in relation to those of vitamin D compounds with very different calcemic activities and pharmacokinetics. The rapid metabolism of calcipotriol probably accounted for its reduced effect on calcium metabolism. In support of this conclusion, the oral $AUC_{0-\infty}$ observed for calcitriol [3690 (pg · hr)/mL] was approximately 100-fold higher than that of an equal dose of calcipotriol [25.3 (pg · hr)/mL] while a similar 80-fold difference was observed in the oral ED_{50} for calcium excretion (calcitriol, 0.7 μg/kg/day; calcipotriol, ~55 μg/kg/day). In contrast to the observations with calcipotriol, a comparison between calcitriol and 1α,24$(OH)_2D_2$ revealed only a 5- to 6-fold difference in oral $AUC_{0-\infty}$ [calcitriol, 3690 (pg · hr)/mL; 1α,24$(OH)_2D_2$, 660 (pg · hr)mL], but almost a 30-fold difference in oral ED_{50} for calcium excretion (calcitriol, 0.7 μg/kg/day; 1α,24$(OH)_2D_2$, ~20 μg/kg/day). These data indicate that the calcemic activity of oral 1α,24$(OH)_2D_2$ is considerably less than its systemic exposure would suggest.

The dose-related effects of calcitriol and calcipotriol on calcium excretion presented in this report are generally consistent with those previously reported by Binderup [17]. Two additional vitamin D analogs studied by Binderup were 1α-OH-D_3 and 1α,24$(OH)_2D_3$, both of which had dose–response curves similar to calcitriol, and not calcipotriol. By extrapolating from these data, 1α,24$(OH)_2D_2$ appears to have much less effect on calcium excretion than 1α-OH-D_3 and 1α,24$(OH)_2D_3$, suggesting that the 1α-24-dihydroxylated vitamin D_2 has less calcemic activity in mammals than its vitamin D_3 counterpart.

While the effect of 1α,24$(OH)_2D_2$ on calcium metabolism was much lower than that predicted from its pharmacokinetics and VDR binding, 1α,24$(OH)_2D_2$ nevertheless exhibits a capacity to produce systemic effects through its reduction of endogenous calcitriol. A reduction of endogenous calcitriol by analogs of vitamin D has been reported previously [8, 20], and presumably occurs through inhibition of the renal 25-hydroxyvitamin D-1-hydroxylase. By this measurement, 1α,24$(OH)_2D_2$ was approximately six times more potent than calcipotriol, and both compounds depressed blood levels of endogenous calcitriol even when undetectable in the circulation (Table 5). Under certain conditions, the blood levels of both endogenous calcitriol and the test analogs were close to the analytical detection limit (5–10 pg/mL).

* Mawer EB, Davies M, Still PE, Jones G, Knutson JC and Bishop CW, 1,24(S)-Dihydroxyvitamin D_2, a biologically active analogue of vitamin D_2, is a naturally occurring metabolite in humans. Abstract, Bone and Tooth Society Meeting, 1995, Warwick, U.K.

Our data indicate that the metabolism of 1α-OH-D_2 to 1α,24$(OH)_2D_2$ accounts, at least in part, for the lower toxicity of 1α-OH-D_2 relative to 1α-OH-D_3. At higher doses of drug, the metabolism to 1α,24$(OH)_2D_2$ increased, while this altered metabolism of 1α-OH-D_2 was not observed for 1α-OH-D_3, even at high dosages. With the formation of 1α,24$(OH)_2D_2$, the calcemic side-effects of high levels of 1α-OH-D_2 were diminished, while other systemic actions were preserved. The properties of 1α,24$(OH)_2D_2$ also indicate that this metabolite itself has therapeutic potential for treating hyperproliferative diseases such as psoriasis and various cancers.

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