Comparison of Effects of 1α-Hydroxy-Vitamin D_3 and 1,25-Dihydroxy-Vitamin D_3 in Man

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ABSTRACT The effects of short-term treatment with 1,25-dihydroxy-vitamin D_3 [1,25(OH)$_2D_3$] or 1α-hydroxy-vitamin D_3 [1α(OH)D_3] on intestinal absorption of ^{47}Ca were compared in 41 experiments in normals and 72 experiments in patients with chronic renal failure. 11 patients were studied a second time after treatment for 2–5 mo. Doses varied from 0.14 to 5.4 μg/day to establish dose-response relationships. Urinary calcium was monitored in normal subjects, nine of whom received a constant calcium intake on a metabolic unit. There was an increase in intestinal absorption of ^{47}Ca and urinary calcium in normals receiving 1,25(OH)$_2D_3$, 0.14 μg/day or greater, and 0.28 μg/day or greater augmented intestinal absorption of ^{47}Ca in chronic renal failure. In contrast, 2.6 μg/day of 1α(OH)D_3 was required to increase intestinal absorption of ^{47}Ca in both groups. The increase in urinary calcium to maximal levels was delayed during treatment with 1α(OH)D_3, 5–10 days vs. 2–5 days with 1,25(OH)$_2D_3$. Moreover, half times for urinary calcium to decrease to pretreatment levels after stopping treatment were greater after 1α(OH)D_3 (1.5–2.7 days) than 1,25(OH)$_2D_3$ (1.1–2.0 days). With long-term administration there was a progressive increase in intestinal absorption of ^{47}Ca in the patients receiving 1α(OH)D_3; this was not observed with 1,25(OH)$_2D_3$.

The pharmacologic differences between 1α(OH)D_3 and 1,25(OH)$_2D_3$ may be explained by the requirement for 25-hydroxylation of 1α(OH)D_3 before biologic effects occur; at low doses (<1 μg/day), 1α(OH)D_3 competes with vitamin D_3 for 25-hydroxylation. With prolonged treatment or larger doses (>2 μg/day), 1α(OH)D_3 could accumulate and then be hydroxylated resulting in production of higher levels of 1,25(OH)$_2D_3$.

INTRODUCTION

The naturally occurring hormonal form of vitamin D_3, 1,25-dihydroxycholecalciferol [1,25(OH)$_2D_3$],[1] and the synthetic analogue 1α-hydroxy-vitamin D_3 [1α(OH)D_3] have both been shown to be highly active in patients with advanced renal failure (1–7). [The synthesis of 1α(OH)D_3 has been accomplished with greater ease than that of 1,25(OH)$_2D_3$, and, it has been claimed that the former may become more readily available for clinical use.] Zerwekh et al. (8) suggested from data in rachitic chicks that 1α(OH)D_3 must undergo 25-hydroxylation before exerting its effects, while Toffolon et al. (9) proposed that this compound may act directly without subsequent metabolic conversion. The potencies of 1,25-(OH)$_2D_3$ and 1α(OH)D_3 were found to be equal in the vitamin D-deficient rat by Toffolon et al. (9) and in the rachitic chick by Haussler et al. (10), respectively. On the other hand, Holick et al. (11) found 1α(OH)D_3 to be about half as potent as 1,25(OH)$_2D_3$ in vitamin

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[1] *Abbreviations used in this paper:* 1,25(OH)$_2D_3$, 1,25-dihydroxycholecalciferol; 1α(OH)D_3, 1α-hydroxy-vitamin D_3.

D-deficient rats. Information which compares the relative potency and the onset and duration of action of 1,25-$(OH)_2D_3$ and $1\alpha(OH)D_3$ in man is not available. In the present study, the actions of $1\alpha(OH)D_3$ and 1,25-$(OH)_2D_3$ on intestinal calcium absorption were evaluated in normal subjects and in patients with renal failure. The present observations indicate that $1\alpha(OH)D_3$ is less effective than $1,25(OH)D_3$ at lower doses, has a slower onset of maximal effect, and may have a more prolonged duration of action after therapy is discontinued. These data provide indirect evidence that 25-hydroxylation of $1\alpha(OH)D_3$ occurs before the exertion of a biologic effect in man.

METHODS

The effects of treatment for 7–12 days (short-term treatment) with $1\alpha(OH)D_3$ and $1,25(OH)_2D_3$ on intestinal calcium absorption and urinary calcium were studied in 41 experiments in 30 normal volunteers (26 males and 4 females). The ages ranged from 20 to 64 yr, with a mean of 41. 12 studies in 6 subjects were carried out in the Metabolic Unit of the VA Wadsworth Hospital Center; the remaining 29 studies were undertaken in 24 reliable volunteers while they ingested their usual diets at home. 72 studies of the effect of short-term treatment on calcium absorption were carried out in 35 patients with advanced renal failure (32 men and 3 women). The patients ages ranged from 20 to 69 yr, with a mean of 52. Eight patients were being treated triweekly with regular hemodialysis (dialysate calcium, 3.5 meq/liter). Endogenous creatinine clearances in those not requiring treatment with dialysis ranged from 5 to 24 ml/min, with a mean of 11 ml/min. These patients received their usual diets without dietary calcium supplements. Most were receiving multivitamin supplements providing 10 μg (400 IU) vitamin D_2 per day. The uremic patients received aluminum hydroxide gel before and during this study. 17 studies were carried out on the Metabolic Ward and the remainder were done on an outpatient basis. Of the 113 studies currently reported, 35 have been previously reported (20 in normal subjects and 15 in azotemic patients) (2). Each subject was informed of the purpose and nature of the investigation and informed consent was obtained.

During short-term treatment, the $1\alpha(OH)D_3$ or 1,25-$(OH)_2D_3$ was given by mouth each morning in 1.0 ml of 1:1, ethanol:1,2-propanediol for 8–12 days. Intestinal absorption of calcium was measured 2–6 wk before initiation of treatment and on the 7th through 12th day of treatment with the sterol. The daily quantities of each sterol given and the number of subjects treated at each dose are shown in Table I. In 14 studies, the $1,25(OH)_2D_3$ used was chemically synthetized according to the method of Narwid et al. (12), (provided courtesy of Hoffman-LaRoche Inc., Nutley, N. J.); in the remaining studies, the $1,25(OH)_2D_3$ utilized was prepared biosynthetically by previously described methods (13). Since there were no differences between the actions of $1,25(OH)_2D_3$ prepared chemically or biosynthetically, the results are combined. Authentic $1\alpha(OH)D_3$ was prepared from the prohormone as described elsewhere (14).

Six additional studies with $1,25(OH)_2D_3$ and three with $1\alpha(OH)D_3$ were carried out in normal volunteers admitted to the Metabolic Unit while they received constant daily diets according to methods previously reported (15). In these studies, daily urinary calcium was determined before and during treatment, and for periods up to 14 days after the agent had been discontinued, until the urinary calcium had returned to base-line levels. In all studies in the normal volunteers, 24-h urine samples were collected for 3 days before treatment and during the last 3 days of treatment.

Intestinal absorption of ^{47}Ca was studied on two separate occasions in 10 uremic patients during treatment with either $1,25(OH)_2D_3$ or $1\alpha(OH)D_3$ for a more prolonged period. The first study was carried out after 8–12 days of treatment as described above (short-term treatment), and the second measurement of absorption was made after 2–5 mo of therapy (long-term treatment). The intestinal absorption of ^{47}Ca was measured after a 12-h overnight fast by a previously described method (16), using a carrier providing 200 mg of calcium as the gluconate salt. Previous studies had shown that there was little variation in the fractional absorption of ^{47}Ca when the same subject was studied on more than one occasion while ingesting the same diet. Urinary calcium was determined by atomic absorption spectrophotometry. Serum levels of calcium and phosphorus were also measured, but the factors influencing these parameters, such as the type and extent of skeletal disease and the severity of secondary hyperparathyroidism, are beyond the scope of the present report; these results and other clinical observations will form the basis for a subsequent communication. Statistical comparisons were made utilizing either t tests or analysis of variance according to standard techniques (17).

RESULTS

The absolute values for calcium absorption and urinary calcium during the control and treatment periods are shown in Table I. The pretreatment values for ^{47}Ca absorption were lower in uremic patients than in normal subjects, consistent with our previous results (16); normal, 0.29 ± 0.009 (SE) and chronic renal failure, 0.21 ± 0.007. When normal subjects and uremic patients were considered separately, the pretreatment values for ^{47}Ca absorption were not different in any group receiving the separate doses of either $1,25(OH)_2D_3$ or 1α-$(OH)D_3$ (normal subjects, $F = 0.30$; and uremic patients, $F = 0.37$). Similarly there were no differences in pretreatment urinary calcium among the different dosage groups, $F = 0.98$. Because of differences in control ^{47}Ca absorption between normal subjects and uremic patients and because of variation among individual subjects, the effects of $1,25(OH)_2D_3$ or $1\alpha(OH)D_3$ were expressed as the change in fraction of ^{47}Ca absorption from control and treatment. These changes in ^{47}Ca absorption in relation to the dose of either $1,25(OH)_2D_3$ or 1α-$(OH)D_3$ are shown in Figs. 1 and 2 for normal subjects and patients with chronic renal disease, respectively. In normal subjects, 0.14 μg/day of $1,25(OH)_2D_3$ increased ^{47}Ca absorption, while 0.65 μg/day of $1\alpha(OH)D_3$ had no significant effect. Moreover, the change in ^{47}Ca absorption with $1,25(OH)_2D_3$, 0.68 μg/day, was significantly greater than the change with $1\alpha(OH)D_3$, 0.65 μg/day ($P < 0.02$). There was no difference between the effects

TABLE I

Mean Values for Fractional ^{47}Ca Absorption in Normal Subjects and Uremic Patients and for Urinary Calcium in Normal Subjects Treated with 1,25(OH)$_2$D$_3$ or 1α(OH)D$_3$

Daily dose	nmol/day	0.065	0.325	0.65	1.625	6.5	13
	μg/day 1,25(OH)$_2$D$_3$*	0.027	0.14	0.28	0.68	2.7	5.4
	μg/day 1α(OH)D$_3$	ND	0.135	ND	0.65	2.6	5.2
				Fraction of ^{47}Ca absorbed (mean±SE)			
Normal							
Control							
1,25(OH)$_2$D$_3$		0.30±0.025 (5)‡	0.28±0.013 (9)	—	0.27±0.018 (6)	0.29±0.031 (5)	—
1α(OH)D$_3$		—	—	—	0.29±0.020 (7)	0.29±0.023 (7)	—
Treatment							
1,25(OH)$_2$D$_3$		0.34±0.022 (4)	0.35±0.013 (9)	—	0.36±0.034 (6)	0.41±0.028 (5)	—
1α(OH)D$_3$		—	—	—	0.30±0.025	0.46±0.037	—
Chronic renal failure							
Control							
1,25(OH)$_2$D$_3$		—	0.22±0.019 (10)	0.17±0.020 (4)	0.20±0.017 (12)	0.21±0.020 (7)	0.21±0.038 (3)
1α(OH)D$_3$		—	0.23±0.020 (4)	—	0.21±0.014 (9)	0.21±0.016 (9)	0.20±0.028 (5)
Treatment							
1,25(OH)$_2$D$_3$		—	0.23±0.013 (10)	0.20±0.022 (4)	0.32±0.028 (12)	0.43±0.030 (7)	0.52±0.065 (3)
1α(OH)D$_3$		—	0.21±0.028 (4)	—	0.23±0.015 (9)	0.35±0.024 (9)	0.53±0.020 (5)
				Urinary calcium excretion (mean±SE)			
				mg/day			
Normal							
Control							
1,25(OH)$_2$D$_3$		158±14 (5)	158±14 (5)	195±25 (8)	—	175±28 (7)	174±24 (5)	—
1α(OH)D$_3$		—		—	—	141±26 (8)	207±30 (7)	—
Treatment							
1,25(OH)$_2$D$_3$		177±23 (5)	177±23 (5)	255±25 (8)	—	275±41 (7)	451±48 (5)	—
1α(OH)D$_3$		—		—	—	174±33 (8)	527±31 (7)	—

* At equivalent nanomole doses, the microgram quantities differ because of differences in molecular weight of 1α(OH)D$_3$ and 1,25(OH)$_2$D$_3$, i.e., 400 and 416, respectively.

‡ The numbers in parentheses indicate the number of studies.

of 2.6–2.7 μg/day of the two sterols. The minimum quantity of 1,25(OH)$_2$D$_3$ necessary to augment absorption in patients with renal disease was 0.28 μg/day, whereas 1α(OH)D$_3$ in a dose of 0.65 μg/day failed to augment ^{47}Ca absorption. Also, the increment in ^{47}Ca absorption was significantly greater in patients receiving 1,25-(OH)$_2$D$_3$ in a dose of 0.68 μg/day than with the equimole dose of 1α(OH)D$_3$ of 0.65 μg/day ($P < 0.01$). A similar increase in ^{47}Ca absorption was observed after administration of either agent at daily doses of 2.6 or 2.7 μg/day.

The changes in urinary calcium excretion paralleled those of calcium absorption in the normal subjects (Fig. 3). The administration of 0.14 μg/day of 1,25(OH)$_2$D$_3$ significantly enhanced urinary calcium excretion, whereas 0.65 μg/day of 1α(OH)D$_3$ had no effect; 2.6–2.7 μg/day of the two compounds augmented urinary calcium to a similar degree. As with the change in ^{47}Ca absorption, the increment in urinary calcium was greater with 1,25-(OH)$_2$D$_3$, 0.68 μg/day, than with 1α(OH)D$_3$, 0.65 μg/day ($P < 0.02$).

Daily measurements of urinary calcium in normal subjects receiving a constant dietary intake of calcium and 0.68–2.7 μg/day of 1,25(OH)$_2$D$_3$ or 2.6 μg/day of 1α-(OH)D$_3$ may provide information about the appearance of a maximal effect of the sterol. With 1,25(OH)$_2$D$_3$, urinary calcium rose quickly and reached a plateau by the 2nd to 5th day of treatment. On the other hand, urinary calcium excretion continued to increase for as long as 6–9 days during treatment with 1α(OH)D$_3$

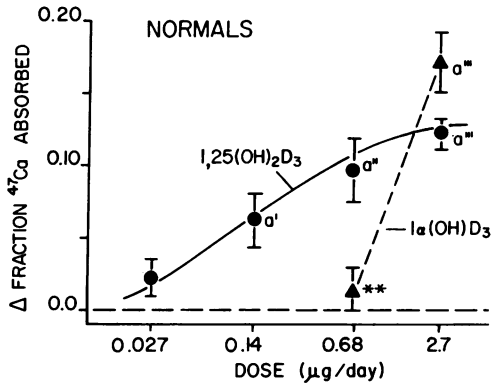

FIGURE 1 Relationship between the daily dose of either 1,25(OH)$_2$D$_3$ (●) and 1α(OH)D$_3$ (▲) and the changes in fraction of ^{47}Ca absorbed in normal subjects. Results are expressed as mean±1 SE; the symbols, a^{I}, a^{II}, and a^{III}, indicate values that are statistically different from control with P values of < 0.02, < 0.01, and < 0.001, respectively. (**) indicates a significant difference between the change in ^{47}Ca absorption with 1,25(OH)$_2$D$_3$ and 1α(OH)D$_3$ ($P < 0.02$).

(Fig. 4). When treatment is discontinued in subjects receiving a constant diet, the decrement in urinary calcium towards the pretreatment rates of excretion may indicate the rate of decay of biologic response to the sterol. The differences in urinary calcium above the mean pretreatment values are shown in relationship to

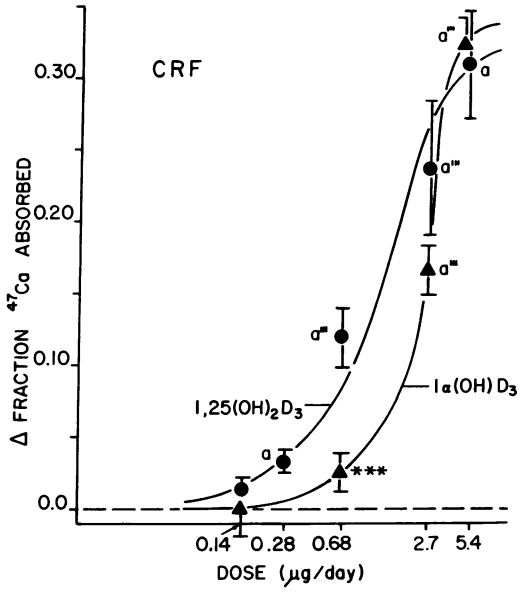

FIGURE 2 Relationship between the daily dose of 1,25-(OH)$_2$D$_3$ (●) and 1α(OH)D$_3$ (▲) and the change in fraction of ^{47}Ca absorbed in patients with chronic renal failure (CRF). Data are expressed as in Fig. 1; "a" indicates a value significantly different from base line with $P < 0.05$; the designation, ***, indicates a difference between the increment in ^{47}Ca absorption with 1,25(OH)$_2$D$_3$ and 1α(OH)D$_3$ ($P < 0.01$).

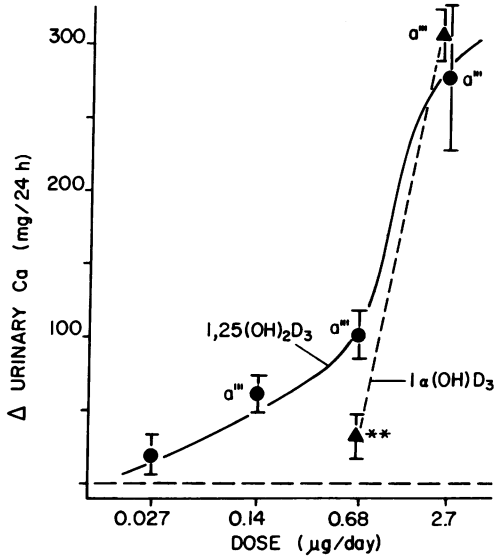

FIGURE 3 Relationship between changes in urinary calcium excretion (Δ urinary Ca in milligrams per 24 h) and the daily doses of 1,25(OH)$_2$D$_3$ (●) and 1α(OH)D$_3$ (▲) in normal subjects. The data and symbols are expressed as in Figs. 1 and 2.

the time after stopping treatment in Figs. 5 and 6. In the subjects receiving 1,25(OH)$_2$D$_3$, urinary calcium fell on the first day with no treatment, and the change approximated a one-component, exponential decay; the half-life ($t_{\frac{1}{2}}$) for disappearance of effect on urinary calcium varied from 1.5 to 2.7 days (mean 1.5±0.15 days). In subjects treated with 1α(OH)D$_3$, urinary calcium did not fall until 2–4 days after the last day of therapy; and the subsequent $t_{\frac{1}{2}}$ for decay averaged 2.2±0.24 days, a value greater than that with 1,25(OH)$_2$D$_3$ ($P < 0.05$). Two subjects (A and B in Figs. 5 and 6) received both compounds; in A, the values for $t_{\frac{1}{2}}$ were 1.3 and 2.1 days with 1,25(OH)$_2$D$_3$ and 1α(OH)D$_3$, respectively; similar values in subject B for $t_{\frac{1}{2}}$ were 2.0 and 2.7 days, respectively.

The results of intestinal absorption of ^{47}Ca in azotemic patients after short-term and long-term treatment with 1,25(OH)$_2$D$_3$ or 1α(OH)D$_3$ are shown in Fig. 7. The change in fraction of ^{47}Ca absorption between the study at 8–12 days (short-term) and 2–5 mo (long-term) of treatment with 1,25(OH)$_2$D$_3$ averaged 0.017± 0.026, a value significantly less than the increment in absorption of 0.34±0.12 with 1α(OH)D$_3$ between 8–12 days and 2–5 mo ($P < 0.02$).

DISCUSSION

The present data verify previous reports of the marked potency of both 1,25(OH)$_2$D$_3$ (1–3) and 1α(OH)D$_3$ (4–7) in patients with renal disease. The results also confirm that 1α(OH)D$_3$ (18) and 1,25(OH)$_2$D$_3$ (2) can augment the intestinal absorption of calcium in nor-

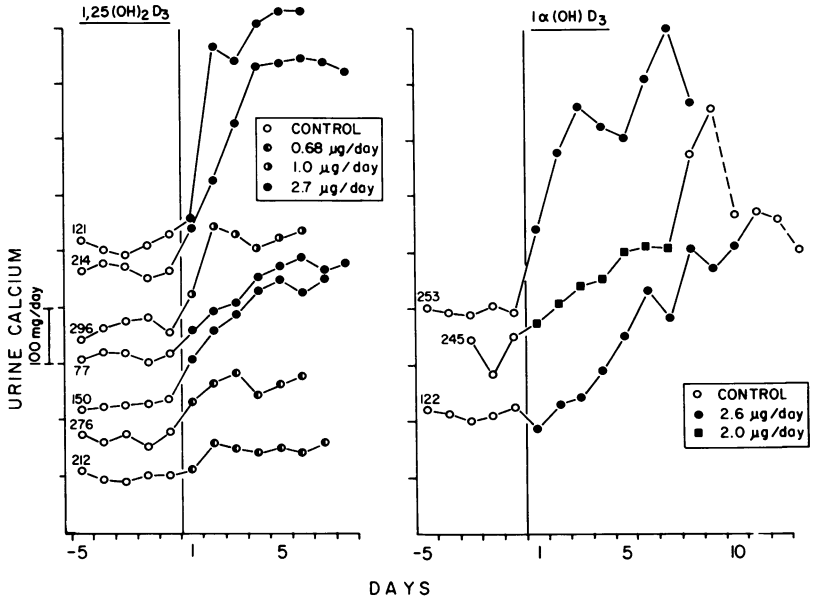

FIGURE 4 Changes in urinary calcium in normal subjects receiving either $1,25(OH)_2D_3$ or $1\alpha(OH)D_3$. (○) represent pre- or post-treatment values. For clarity, the rate of calcium excretion is plotted arbitrarily on the ordinate with the value on the first control day in milligrams per day shown for each subject.

mal man. Also a dose-response relationship for 1α-$(OH)D_3$ in uremic patients is reported together with additional information about dose-response relationships of $1,25(OH)_2D_3$ in normal subjects and patients with renal failure. Responses of normal subjects to two doses of $1\alpha(OH)D_3$ is also reported.

The present observations in man demonstrate several differences between the pharmacologic properties of

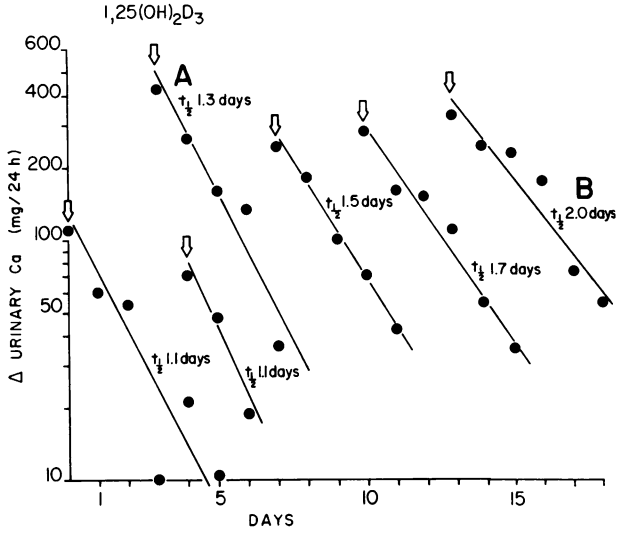

FIGURE 5 Dissipation of biological effect of $1,25(OH)_2D_3$ as measured by the return of urinary calcium excretion toward pretreatment levels in normal subjects. The changes in urinary calcium (Δ urinary Ca), shown on a $\log_{10}$ scale, represent the difference between the observations on each post-treatment day and the mean pretreatment rate of excretion. The regression lines were calculated by the method of least squares (17). The arrows indicate the last day of treatment with the sterol. The designations, A and B, refer to subjects who received both $1,25(OH)_2D_3$ and $1\alpha(OH)D_3$ (Fig. 6).

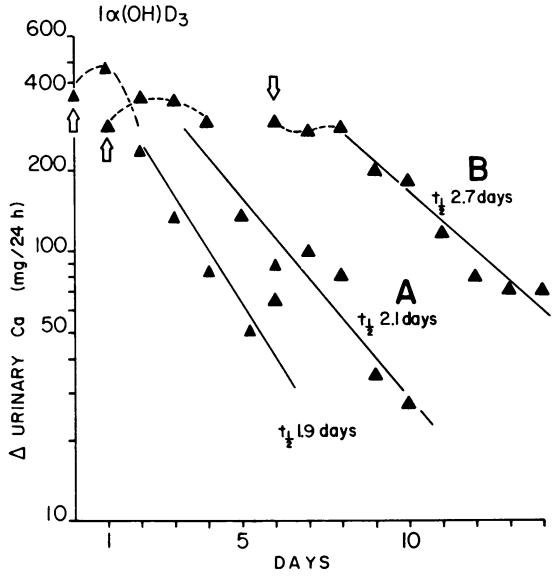

FIGURE 6 Dissipation of biological effect of $1\alpha(OH)D_3$, as measured by the return of urinary calcium excretion toward pretreatment levels, in normal subjects. The data are shown as in Fig. 5.

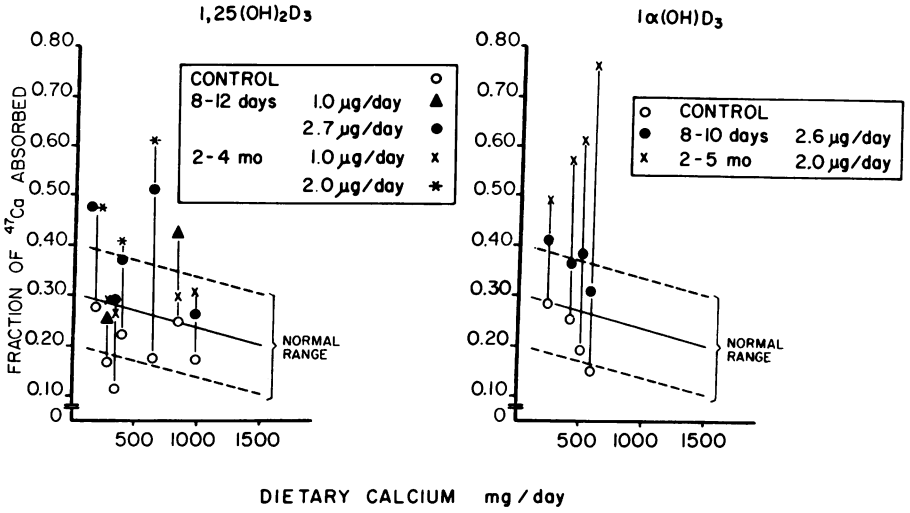

FIGURE 7 Fractional absorption of ^{47}Ca, expressed in relationship to previous habitual dietary calcium intake in uremic subjects treated with either 1,25(OH)$_2$D$_3$ or 1α(OH)D$_3$. Studies were carried out after treatment for 8–12 days and again at 2–5 mo. (——) connect observations made in the same patient. The range of absorption observed in normal subjects (- - - -) has been reported elsewhere (16).

1,25(OH)$_2$D$_3$ and 1α(OH)D$_3$; thus, 1α(OH)D$_3$ is less potent than 1,25(OH)$_2$D$_3$ at doses below 1 μg/day, but equally potent at doses above 2.0 μg/day. The onset of maximal action, as judged by the increment in urinary calcium, is more gradual with 1α(OH)D$_3$ than 1,25-(OH)$_2$D$_3$, and the duration of effect disappeared at a slower rate after 1α(OH)D$_3$ was discontinued than observed with 1,25(OH)$_2$D$_3$. In addition, observations in azotemic patients given the compounds for several months suggest that there may be a progressive or cumulative effect of 1α(OH)D$_3$ compared to 1,25(OH)$_2$D$_3$. The last observation is similar to that of Catto et al. (5), who noted a progressive increase in calcium absorption in two of three uremic patients receiving a constant dose of 1α(OH)D$_3$ for 9–10 wk.

Our observations in man of the relative potencies of 1α(OH)D$_3$ and 1,25(OH)$_2$D$_3$ are in agreement with results of Holick et al. (19), who gave rats either 1α(OH)D$_3$ or vitamin D$_3$ daily for 7 days. The 1α-(OH)D$_3$ was 2–5 times more potent than vitamin D$_3$ compared to their previous data showing 1,25(OH)$_2$D$_3$ to be 5–10 times more potent than vitamin D$_3$ (20).

In contrast, other data obtained in vitamin D-deficient rats and chicks have indicated that 1α(OH)D$_3$ is at least as potent as 1,25(OH)$_2$D$_3$; Haussler et al. (10), and Cork et al. (21) found 1α(OH)D$_3$ to be equally or slightly more active than 1,25(OH)$_2$D$_3$ in chicks. Toffolon et al. (9) found the two sterols to be equally effective in stimulating calcium transport in very young rats, while Pechet and Hesse reported that 1α(OH)D$_3$ was more effective than 1,25(OH)$_2$D$_3$ in mobilizing skeletal calcium in rats (22). Toffolon et al. reported 1α(OH)D$_3$ and 1,25(OH)$_2$D$_3$ to have equally rapid appearance of action after a single dose; on the basis of these data, they suggested that 1α(OH)D$_3$ acted directly without subsequent metabolic conversions (9).

A number of studies comparing the effects of 1α-(OH)D$_3$ and 1,25(OH)$_2$D$_3$ in vitro support the contention that 1α(OH)D$_3$ must undergo metabolic conversion, presumably to 1,25(OH)$_2$D$_3$, before exerting its effect. Thus, Procsal et al. (23) found 1α(OH)D$_3$ to bind 1/800th as avidly to the chick intestinal-cytosol-chromatin receptor system for 1,25(OH)$_2$D$_3$, as did 1,25(OH)$_2$D$_3$, itself; Zerwekh et al. (8) found the binding affinity of 1α(OH)D$_3$ to be 1/100th to 1/1,000th as great as 1,25-(OH)$_2$D$_3$. Since the two sterols were equally active in vivo, it was suggested that 1α(OH)D$_3$ undergoes rapid 25-hydroxylation to 1,25(OH)$_2$D$_3$ before exerting its action. Moreover, only 1,25(OH)$_2$D$_3$, as identified by the competitive binding assay, was found associated with chick intestinal chromatin after treating intact birds with 1α(OH)D$_3$ (8). Reynolds et al. (24) found 1α-(OH)D$_3$ to be 1/100th as active as 1,25(OH)$_2$D$_3$ in promoting bone resorption when the compounds were added to explants of mouse calvaria, in vitro. In contrast, the compounds were nearly equal in potency when they were given in vivo before the evaluation of bone resorption, in vitro.

The duration of action of 1α(OH)D$_3$ and 1,25-(OH)$_2$D$_3$ has received little attention in animal studies. Haussler et al. (10) found 1α(OH)D$_3$ and 1,25(OH)$_2$D$_3$ to be equally active 40 h after their administration to chicks, and Toffolon et al. found the agents to have similar actions 14 h after giving a single dose to very

young rats (9). The present studies in normal man indicate both a delayed appearance of maximal action after the daily administration of 1α(OH)D_3 and a slower dissipation of effect after the cessation of therapy than were observed with 1,25(OH)$_2D_3$.

The observations of potency and of onset and duration of action of 1α(OH)D_3 compared to 1,25(OH)$_2D_3$ in man may be explained by the necessity that 1α(OH)D_3 must undergo 25-hydroxylation to 1,25(OH)$_2D_3$ before exerting its effect. Both normal subjects and uremic patients would be expected to have adequate quantities of vitamin D_3, and exogenous 1α(OH)D_3 and endogenous vitamin D_3 may compete for the same hepatic (or intestinal [8]) 25-hydroxylase. Our suggestion of a requirement in man for hepatic hydroxylation of 1α(OH)D_3 before the onset of its biological action is consistent with the recent report of Fukushima et al. (25). They obtained unequivocal evidence that radioactive 1α(OH)D_3 was rapidly converted to 1,25(OH)$_2D_3$ in an isolated, perfused rat liver system.

With treatment of patients with low doses of 1α(OH)D_3 or during the first few days of therapy, 25-hydroxylation of vitamin D_3 may be favored over that of 1α(OH)D_3. Hence, 1α(OH)D_3 is less potent than 1,25(OH)$_2D_3$. During continued treatment with 1α(OH)D_3 or with the administration of larger doses, its plasma or tissue level may increase, and sufficient 25-hydroxylation may occur to produce the resultant biologic action. The augmentation of ^{47}Ca absorption observed after continued long-term treatment with 1α(OH)D_3 could be accounted for by the accumulation of increased body stores of the sterol and its continued 25-hydroxylation. Moreover, the 25-hydroxylation of 1α(OH)D_3 probably occurs continuously with persistent production of 1,25(OH)$_2D_3$. The turnover rates of plasma and tissue pools of 1,25(OH)$_2D_3$ are probably quite rapid (26), and it is likely that wide fluctuations of plasma and tissue concentrations of 1,25(OH)$_2D_3$ occur during treatment with single daily doses of exogenous sterol.

The observation that 1α(OH)D_3 produces a rapid action and has the same potency as 1,25(OH)$_2D_3$ in vitamin D-deficient rats (9) or chicks (10) after the administration of a single dose may be explained by a greater activity of 25-hydroxylase in the vitamin D-deficient state, or because there is no vitamin D_3 present to compete for the cholecalciferol-25-hydroxylase. On the other hand, it should be pointed out that both 1,25(OH)$_2D_3$ and 1α(OH)D_3 are effective in man at doses that are at least two orders of magnitude lower than those used in the rat (9) or chick (10).

The present study was not carried out to compare the therapeutic efficacy or safety of 1,25(OH)$_2D_3$ and 1α(OH)D_3. However, the progressive increase in ^{47}Ca absorption in uremic patients receiving 1α(OH)D_3 for several months, both in the present study and in that of Catto et al. (5), suggests that a danger of "vitamin D intoxication" could exist during prolonged treatment with 1α(OH)$_3$. Clearly, there is a need for further observations of the long-term actions of both 1α(OH)D_3 and 1,25(OH)$_2D_3$ before either agent enters widespread clinical use.

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