Evidence that 1α-Hydroxy-Vitamin D₃ is Converted to 1,25-Dihydroxy-Vitamin D₃ Before Acting in Normal and Uraemic Man

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Summary

1,25-dihydroxy-vitamin D₃ (1,25(OH)₂D₃ and 1α-hydroxy-vitamin D₃ (1α(OH)D₃) in doses varying between 0.065 to 13 nmoles (0.027 to 5.4 μg/day) were given to normal subjects and uraemic patients to establish dose-response relationships. Also urinary calcium was measured in normal subjects. The data indicate that 1α(OH)D₃ is less effective than 1,25(OH)₂D₃ at lower doses in both normal subjects and uraemic patients. Also, 1α(OH)D₃ exhibits a slower onset of maximal effect and extends its action for a more prolonged time after therapy is discontinued. The data provide indirect evidence that 25-hydroxylation of 1α(OH)D₃ occurs before the latter exerts a biologic effect in man.

Introduction

Both 1,25-dihydroxy-vitamin D₃ (1,25(OH)₂D₃), the naturally occurring sterol, and its synthetic analogue 1α-hydroxy-vitamin D₃ (1α(OH)D₃) are highly active in patients with uraemia (Brickman et al, 1972; Chalmers et al, 1973). Certain data obtained in experimental animals suggest that 1α(OH)D₃ must be hydroxylated to 1,25(OH)₂D₃ before the former acts (Zerwekh et al, 1974; Fukushima et al, 1975); other observations (Toffolon et al, 1975) suggest that 1α(OH)D₃ may act directly. To date, comparative observations of the actions of these sterols in man are not available. Since both have been synthesised and may be available for widespread use, such data may be of considerable interest. In this study, the actions of 1α(OH)D₃ and 1,25(OH)₂D₃ on intestinal calcium absorption were evaluated in normal subjects and patients with renal failure.
MATERIAL AND METHODS

The short-term effects of 1α(OH)D₃ and 1,25(OH)₂D₃ on intestinal calcium absorption were studied in 41 experiments in normal subjects and 72 studies in patients with advanced renal failure. Urinary calcium excretion was measured in the normal subjects. All subjects received their usual diets without calcium supplements, and most of the uraemic patients were also given aluminium hydroxide gel before and during the study. Twenty-nine studies were carried out on the metabolic ward, and the remainder were done on an outpatient basis. Each subject was informed of the purpose and nature of the investigation and a form of consent was obtained.

The 1α(OH)D₃ and 1,25(OH)₂D₃, suspended in 1:1, ethanol:1,2-propanediol, were given by mouth for 8 to 12 days. Intestinal absorption of ⁴⁷Ca was measured 2 to 6 weeks before initiation of treatment and again after 7 to 12 days of treatment with the sterol. The daily quantities of 1,25(OH)₂D₃ given varied from 0.065 to 6.5 nmols/day (0.027 to 2.7 μg), while the doses of 1α(OH)D₃ varied from 0.325 to 13 nmols/day (0.13 to 5.2 μg). In 14 studies, the 1,25(OH)₂D₃ used was chemically synthesised (Narwid et al, 1974) and provided by courtesy of Dr M Uuskokovic, Hoffman LaRoche Co, Nutley, New Jersey; in the other studies using 1,25(OH)₂D₃, the latter was prepared biosynthetically (Norman et al, 1971). The actions of chemically and biosynthetically prepared 1,25(OH)₂D₃ were similar, and the results have been combined. Authentic 1α(OH)D₃ was prepared from the prohormone (Mitra et al, 1974).

Ten uraemic patients were treated with either 1,25(OH)₂D₃ or 1α(OH)D₃ for more prolonged periods, and intestinal absorption of ⁴⁷Ca was studied on two or more separate occasions. The first study was carried out after 8 to 12 days of treatment as described above (short-term study), and the subsequent measurements of absorption were made after 1.5 to 5 months of therapy (long-term treatment). In all studies, the intestinal absorption of ⁴⁷Ca was measured after an overnight fast as previously described (Coburn et al, 1972).

RESULTS

Figure 1 shows the increment in ⁴⁷Ca absorption in the uraemic patients in relation to the daily dose of 1,25(OH)₂D₃ or 1α(OH)D₃ given. The minimum quantity of 1,25(OH)₂D₃ which significantly augmented absorption of ⁴⁷Ca was 0.65 nmols/day, whereas 1α(OH)D₃ did not augment absorption in a dose of 1.625 nmols/day. Similar increments in ⁴⁷Ca absorption were noted following the administration of either agent at daily doses of 6.5 nmols or more. In the normal subjects, 0.325 nmols/day of 1,25(OH)₂D₃ increased ⁴⁷Ca absorption, while 1.625 nmols of 1α(OH)D₃ had no discernible effect. On the other hand, there was no difference between the actions of the two sterols at a dose of 6.5 nmols/day.
Figure 1. The relationship between the daily doses of $1,25(\text{OH})_2\text{D}_3$ (●) and $1\alpha(\text{OH})\text{D}_3$ (▲) and the changes in fraction of $^{47}\text{Ca}$ absorbed in uraemic patients with chronic renal failure. The results are expressed as mean ± 1 SE. With a dose of 1.625 nmoles/day, the increment following $1,25(\text{OH})_2\text{D}_3$ was significantly greater than that for $1\alpha(\text{OH})\text{D}_3$ ($p < 0.01$).

Figure 2. Changes in urinary excretion of calcium in a normal subject who received similar amounts of $1,25(\text{OH})_2\text{D}_3$ and $1\alpha(\text{OH})\text{D}_3$ (6.5 nmole/day). The cross-hatched area indicates the period of treatment. The subject was equilibrated on a constant diet for 5 days before the initiation of the study.
The increments in urinary calcium excretion paralleled the increases in calcium absorption in the normal subjects. A quantity of 0.325 nmoles/day of 1,25(OH)₂D₃ enhanced urinary calcium excretion, while 1.625 nmoles/day had no effect; 6.5 nmoles/day of the two agents augmented urinary calcium to a similar extent.

The increments in urinary calcium in normal subjects receiving a constant dietary calcium intake with effective quantities of 1,25(OH)₂D₃ or 1α(OH)D₃ may indicate the time of appearance of the maximal effect of the sterol. With the administration of 1,25(OH)₂D₃, urinary calcium rose rapidly and plateaued by the 3rd or 4th day of treatment. In contrast, urinary calcium excretion continued to increase for as long as 5 to 10 days during treatment with 1α(OH)D₃ (Figure 2). After treatment is stopped in normal subjects receiving a constant dietary intake, the decrement in urinary calcium toward the pre-treatment rate of excretion provides a measure of the rate of decay of the biological action of the sterol. In the normal subjects taking 1,25(OH)₂D₃, urinary calcium fell on the first day with no treatment, and the change approximated a one-component, exponential decay; the half-time (t½) for disappearance of the effect on urinary calcium varied from 1.5 to 2.7 days in this group with a mean of 1.5 ± 0.15 days. In subjects that had received 1α(OH)D₃, urinary calcium did not fall until 2 to 4 days after therapy was stopped, and the subsequent t½ for decay averaged 2.2 ± 0.24 days, a value greater than that observed with 1,25(OH)₂D₃ (p<0.05).

Figure 3. Changes in ⁴⁷Ca absorption in related to the duration of long-term therapy with 1,25(OH)₂D₃ or 1α(OH)D₃ in uraemic patients.
The changes in intestinal absorption of $^{47}$Ca in the azotaemic patients in relation to the duration of treatment with 1,25(OH)$_2$D$_3$ or 1α(OH)D$_3$ are shown in Figure 3. With 1,25(OH)$_2$D$_3$, there was an increment in $^{47}$Ca absorption at 7-10 days that did not increase further with continued treatment; in contrast, there was a continued augmentation in $^{47}$Ca absorption in the patients given 1α(OH)D$_3$.

DISCUSSION

Our current data verify previous reports of the marked potency of 1,25(OH)$_2$D$_3$ and 1α(OH)D$_3$ in uraemic patients and in normal men. Several differences between the pharmacologic actions however, of 1,25(OH)$_2$D$_3$ and 1α(OH)D$_3$ have been observed: thus, 1α(OH)D$_3$ is less potent than 1,25(OH)$_2$D$_3$ at daily doses less than 5 nmoles/day. The increment in urinary calcium, an indication of onset of maximal action, occurs more gradually with 1α(OH)D$_3$. In addition, there may be a progressive or cumulative effect of 1α(OH)D$_3$ compared to 1,25(OH)$_2$D$_3$ in azotaemic patients given either compound for several months. Catto et al (1975) also observed a progressive increase in calcium absorption in 2 of 3 uraemic patients given a constant dose of 1α(OH)D$_3$ for 9-10 weeks.

Our observations in man of the relative potencies of 1α(OH)D$_3$ and 1,25(OH)$_2$D$_3$ agree with results of Holick et al (1975). In contrast, others have reported that 1α(OH)D$_3$ is as potent as 1,25(OH)$_2$D$_3$ in vitamin D-deficient rats and chicks (Haussler et al, 1973; Cork et al, 1974).

Other studies comparing the effects of 1α(OH)D$_3$ and 1,25(OH)$_2$D$_3$ in vitro support a view that 1α(OH)D$_3$ must undergo metabolic conversion to 1,25(OH)$_2$D$_3$ prior to exerting its effect. Thus, Procsal et al (1975) and Zerwekh et al (1974) found 1α(OH)D$_3$ to bind to the chick intestinal-cytosol-chromatin receptor system for 1,25(OH)$_2$D$_3$ less avidly than did 1,25(OH)$_2$D$_3$ itself. Also Reynolds et al (1974) found 1α(OH)D$_3$ to be 1/100th as active as 1,25(OH)$_2$D$_3$ in promoting bone resorption by explants of mouse calvaria.

The present observations of potency, onset and duration of action of 1α(OH)D$_3$ compared to 1,25(OH)$_2$D$_3$ in man may be explained by the necessity that 1α(OH)D$_3$ must undergo 25-hydroxylation to 1,25(OH)$_2$D$_3$ before exerting its effect. Normal subjects and uraemic patients would have adequate quantities of vitamin D$_3$; and exogenous 1α(OH)D$_3$ and endogenous vitamin D$_3$ may compete for the same 25-hydroxylase. Our suggestion of a requirement in man for hepatic hydroxylation of 1α(OH)D$_3$ prior to the onset of its biological action is consistent with the recent report of Fukushima et al (1975), who found that radioactive 1α(OH)D$_3$ was rapidly converted to 1,25(OH)$_2$D$_3$ by the isolated perfused rat liver.

With use of low doses of 1α(OH)D$_3$ or during the first few days of therapy, the 25-hydroxylation of vitamin D$_3$ may be favoured over that of 1α(OH)D$_3$. Hence, 1α(OH)D$_3$ would be less potent than 1,25(OH)$_2$D$_3$. 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 result in greater biological action. Augmented $^{47}$Ca absorption following continued,
long-term treatment with 1α(OH)D₃ could be accounted for by the accumulation of increased body stores of 1α(OH)D₃ and its continued 25-hydroxylation. Also, the 25-hydroxylation of 1α(OH)D₃ probably provides continuous production of 1,25(OH)₂D₃ compared to the wide fluctuations of plasma and tissue concentrations of 1,25(OH)₂D₃ that probably occur following single daily doses of 1,25(OH)₂D₃.

The rapid action and high potency of 1α(OH)D₃ in vitamin D-deficient rats or chicks may be explained by the high activity of 25-hydroxylase in the vitamin D-deficient state or because no vitamin D₃ is present to compete for the cholecalciferol-25-hydroxylase.

The present study was not carried out to compare the therapeutic efficiency or safety of 1,25(OH)₂D₃ and 1α(OH)D₃. However, the progressive increase in ⁴⁷Ca absorption in uraemic patients receiving 1α(OH)D₃ for several months suggest that ‘vitamin D intoxication’ may be more likely to develop during long-term treatment with 1α(OH)D₃. Moreover, 1α(OH)D₃ may be far less effective in uraemic patients receiving drugs which alter the hepatic 25-hydroxylation of vitamin D₃ to 25(OH)D₃. There is a need for further observations of the long-term actions of both 1α(OH)D₃ and 1,25(OH)₂D₃ before these agents enter widespread clinical use.

Acknowledgments

Kornel Gerszi, John Lee, Herb Dennin, Robert Adachi, Sonia Soghomonian, June Bishop and Patricia Roberts provided technical assistance, as did the Metabolic Unit nurses, Ann Chance, Shirley MacKay and Nora Hechanova and the Hospital and Metabolic Unit dieticians, Molly Sorensen and Dorothy Mulcare. Iona Gordon provided secretarial assistance.

Supported, in part, by USPHS Grants AM-14750, AM-16595, AM-09102, Contract AM-5-2234 with the USPHS, and VA Research Funds.

AWN is the recipient of Research Career Development Award.

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Open Discussion

KANIS (Oxford) As you know, we have made similar observations in Oxford which support your suggestion of a difference in the pharmacological properties between $1\alpha$-hydroxycholecalciferol ($1\alpha$(OH)D$_3$) and $1\alpha$,25-dihydroxycholecalciferol ($1,25$(OH)$_2$D$_3$). We have also observed that after $1\alpha$(OH)D$_3$-induced hypercalcaemia has been reversed, retreatment with a smaller dose results in an immediate recurrence of hypercalcaemia. This suggests that sensitivity to $1\alpha$(OH)D$_3$ has been altered during previous treatment or that storage of $1\alpha$(OH)D$_3$ has occurred.

Finally I should like to add that the half-time of reversal of D-toxicity using either $1\alpha$(OH)D$_3$ or $1,25$(OH)$_2$D$_3$ is longer in the vitamin-resistant disorders than in normal subjects.

COBURN Thank you for your comments. I was aware of these results from communication with Dr RGG Russell after his recent visit to the US. It is nice that our data are in agreement.

FOURNIER (Amiens) I would like to ask if you have had our experience of hypercalcaemia with $1\alpha$(OH)D$_3$ and $1,25$(OH)$_2$D$_3$. If so what was the delay of recovery to normal plasma calcium levels? I have observed two cases of hypercalcaemia up to 13 mg/dl which recovered within three days after discontinuation of $1\alpha$(OH)D$_3$.

COBURN Among 40 uraemic patients we have treated for long-term periods with either $1,25$(OH)$_2$D$_3$ or $1\alpha$(OH)D$_3$ we had six instances where blood calcium exceeded 14.0 mg/dl; 3 were treated with $1\alpha$(OH)D$_3$ and 3 with $1,25$(OH)$_2$D$_3$. In the patients taking $1,25$(OH)$_2$D$_3$ the serum calcium usually returned to normal within two to three days after stopping. In the patient taking $1\alpha$(OH)D$_3$ three to five were required. The data from the Oxford group just described by Dr Kanis support this. These patients were not hospitalised and we did not obtain blood calcium measurements very frequently.