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Effect of 25-hydroxycholecalciferol on calcium absorption in chronic renal disease

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Effect of 25-hydroxycholecalciferol on calcium absorption in chronic renal disease. Calcium absorption was measured in eight uremic patients before and after eight days of treatment with 100 or 500 μg of 25-hydroxycholecalciferol ($25(OH)D_3$) per day. Fractional calcium absorption was estimated by administering ^{47}Ca i.v. and orally on separate days and counting forearm radioactivity four hours later. Calcium absorption in four patients with residual renal function rose from 16.3 ± 2.5 to 40.8 ± 5.5% after treatment. In order to determine if the increased calcium absorption was mediated by an increase in the production of 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) by virtue of increased substrate delivery to the 25-hydroxycholecalciferol-1-hydroxylase system present in the residual renal tissue, identical studies were performed in four anephric patients. Calcium absorption in these patients averaged 15.7 ± 2.2% during the control period and rose to 46.0 ± 11.1% after treatment. Increments in serum calcium after treatment were similar in both groups of patients; the mean concentration rose from 9.6 ± 0.3 to 11.0 ± 0.6 mg/100 ml. The results indicate that $25(OH)D_3$ can improve calcium absorption in the absence of renal tissue suggesting that its conversion to $1,25(OH)_2D_3$ may not be necessary for its effect on the gastrointestinal tract in the uremic patient.

Effet du 25-hydroxycholécalciferol sur l'absorption du calcium au cours de l'insuffisance rénale chronique. L'absorption de calcium a été mesurée chez huit malades urémiques avant et après huit jours de traitement par 100 ou 500 μg de $25(OH)D_3$ par jour. L'absorption fractionnelle du calcium a été évaluée par l'administration de ^{47}Ca, intraveineuse et orale, à des jours différents, et par comptage de l'activité de l'avant-bras quatre heures après. L'absorption du calcium chez quatre malades ayant une fonction rénale résiduelle augmente de 16,3 ± 2,5 à 40,8 ± 5,5% après traitement. Des études semblables ont été réalisées chez quatre malades anéphriques de façon à apprécier le rôle éventuel, dans l'augmentation de l'absorption intestinale, d'une augmentation de la production de $1,25(OH)_2D_3$ due à l'augmentation de l'apport de substrat à la 25-hydroxycholécalciferol-1-hydroxylase du tissu rénal. L'absorption du calcium chez ces malades est en moyenne de 15,7 ± 2,2% pendant la période contrôle et augmente après traitement à 46,0 ± 11,1%. Les augmentations du calcium sériques sont semblables dans les deux groupes de malades, la concentration moyenne passe de 9,6 ± 3 à 11,0 ± 0,6 mg/100 ml. Les résultats indiquent que $25(OH)D_3$ peut améliorer l'absorption du calcium en l'absence de tissu rénal ce qui suggère que sa conversion en $1,25(OH)_2D_3$ peut ne pas être nécessaire à son action sur le tractus gastro-intestinal du malade urémique.

Calcium malabsorption, hypocalcemia, secondary hyperparathyroidism and renal osteodystrophy are consequences of renal insufficiency [1–6] which appear to be related, at least in part, to an alteration(s) in the metabolism of vitamin D [1, 4, 6]. Vitamin D, per se, has relatively low physiologic activity and must first be metabolized to 25-hydroxycholecalciferol, $25(OH)D_3$, by a liver microsomal enzyme system which in mammals appears to be product inhibitable [7–10]. This inhibition may account for the fact that the circulating concentrations of $25(OH)D_3$ in serum are maintained in the range of 20 to 30 ng/ml in normal subjects and in uremic patients as well [11]. Under the appropriate conditions, $25(OH)D_3$ may undergo further hydroxylation to 1,25-dihydroxycholecaliferol, $1,25(OH)_2D_3$ [12–14]. The enzyme responsible for this process, 25-hydroxycholecalciferol-1-hydroxylase, is present exclusively in the mitochondria of the renal cortex [15]. $1,25(OH)_2D_3$ is much more potent than $25(OH)D_3$ in stimulating calcium absorption and mobilizing calcium from bone [16–18], and it is now generally accepted that calcium malabsorption in uremia is the result of insufficient conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$. Moreover, recent studies by Brickman, Coburn and Norman [19] have clearly demonstrated that the administration of small amounts of $1,25(OH)_2D_3$ to uremic patients reverses the malabsorption of calcium and increases serum calcium concentration in a relatively short period of time.

Previous studies from this laboratory demonstrated that calcium absorption could be normalized in uremic dogs by the oral administration of $25(OH)D_3$ [20]. This observation was of great practical interest because $25(OH)D_3$, unlike $1,25(OH)_2D_3$, is relatively inexpensive and is available experimentally in large quantities. The effect of $25(OH)D_3$ on calcium absorption was also of considerable theoretical interest because calcium absorption has been found to be enhanced by compounds which, in addition to possessing a basic vitamin D-like structure, also generally contain a hydroxyl group in the C-1 position [21, 22].

$25(OH)D_3$ may have normalized calcium absorp-

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tion in our uremic dogs by virtue of the fact that its oral administration effectively bypassed the 25-hydroxylase system in the liver, and permitted the attainment of circulating concentrations of $25(OH)D_3$ in excess of those which normally occur. At this higher concentration the metabolite may be able to stimulate calcium absorption directly or, alternatively, may permit increased production of $1,25(OH)_2D_3$ or other metabolites by virtue of increased delivery of $25(OH)D_3$ to the 1-hydroxylase system in the remaining renal mass.

With this background in mind, the present study was undertaken to measure calcium absorption in uremic patients with residual renal function and in anephric patients before and after eight days of treatment with $25(OH)D_3$.

Methods

Eight patients with chronic renal disease were admitted to the Clinical Research Center at Washington University and fed a constant diet containing 800 mg of calcium and 400 mg of phosphorus. Four of these eight patients were anephric. Surgical removal of the kidneys in these patients had been necessary to control hypertension. Aluminum carbonate gel was administered in a dose of 20 to 30 ml three to four times daily to maintain the serum phosphorus concentration within normal limits. An equilibration period of 10 to 14 days on this diet preceded the control calcium absorption measurements. Following this determination, $25(OH)D_3$ was given orally in a dose of either 500 or 100 μg daily for eight days and calcium absorption was measured again.

Calcium absorption was estimated by a previously described modification [23] of the method of Curtis, Fellows and Rich [24]. The measurements were performed as follows: ^{47}Ca was given by constant i.v. infusion over 90 min and the usual breakfast containing 200 mg of calcium was given at the start of the infusion. Forearm radioactivity was counted in a volume counter (Armac) prior to the administration of isotope and four hours later. Two days later 10 μCi of ^{47}Ca was given orally with the same meal and forearm radioactivity was counted four hours later. Appropriate counting was performed in order to correct for the radioactivity which remained from the previously administered ^{47}Ca. The absorption at 4 hr rather than 24 hr was measured as described by Pak et al [25]. Calcium absorption is expressed as a percent of the orally administered load. Creatinine clearance and serum calcium and phosphorus concentrations were measured by methods previously described [26].

Results

Measurements of calcium absorption demonstrated a marked difference between normal subjects and patients with chronic renal disease. Figure 1 illustrates the results obtained in a group of 5 normal volunteers and 11 uremic patients with a mean glomerular filtration rate of 13.6 ± 3.2 ml/min. Mean calcium absorption was 44.4 ± 3.5% in the normal subjects while the uremic patients absorbed 18.6 ± 3.4% of the orally administered load.

The results of six studies in four patients with a mean creatinine clearance of 2.4 ± 1.1 ml/min on two different dosage schedules of $25(OH)D_3$ are shown in Fig. 2. An interval of approximately two months separated the studies in the patients who received two different doses. Calcium absorption during the control period averaged 16.3 ± 2.5% and rose to 40.8 ± 5.5% after treatment with $25(OH)D_3$, thus confirming in patients our findings in uremic dogs that $25(OH)D_3$ effectively increases calcium absorption in chronic renal disease in a relatively short period of time. The smaller dose (100 μg) also was equally effective.

In order to determine if the improvement in calcium absorption following the administration of $25(OH)D_3$ was mediated by its renal conversion to $1,25(OH)D_3$, identical studies were performed in four anephric patients. All four received 500 μg of $25(OH)D_3$ daily for a period of eight days. Figure 3 shows the results of this study.

Calcium absorption during the control period averaged 15.7 ± 2.2%; it increased to 46.0 ± 11.1% after the administration of $25(OH)D_3$. Calcium absorption increased in all, but was highly variable from patient to patient.

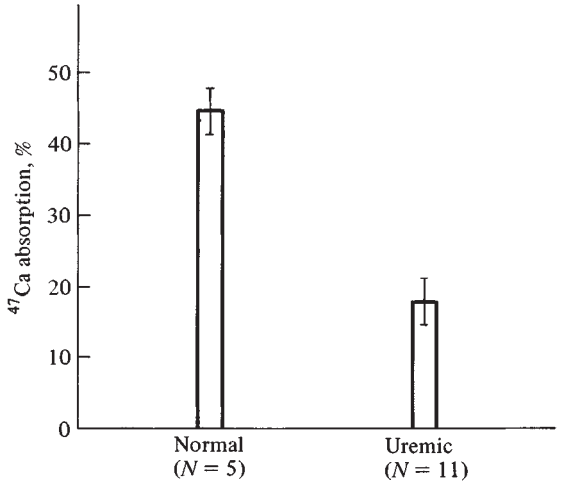

Fig. 1. *Calcium absorption in normal and uremic patients.* Mean results and SEM for 5 normal volunteers and 11 patients with chronic renal disease.

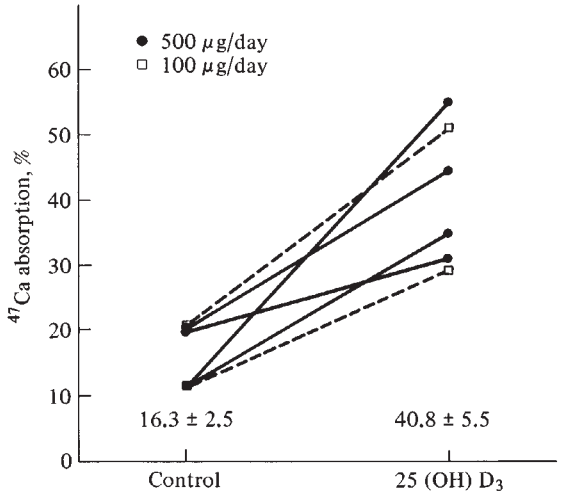

Fig. 2. *Calcium absorption in four patients with chronic renal disease before and after eight days of treatment with 25(OH)D$_3$.* The values for patients who received 500 μg daily are represented by *dark circles* and connected by *solid lines* (●—●); those receiving 100 μg daily are represented by *open squares* and connected by *interrupted lines* (□- - -□). The numbers at the bottom of the figure represent the mean values and SEM for calcium absorption in six studies performed before and after treatment.

Figure 4 depicts the changes in serum calcium concentration before and after eight days of treatment with 500 μg daily of 25(OH)D$_3$ for eight patients with far advanced chronic renal disease (four were anephric). The increment in serum calcium concentration after treatment with 25(OH)D$_3$ was similar in both groups of patients, and for all eight patients, serum calcium concentration rose from a mean of 9.6 ± 0.3 to 11.0 ± 0.6 mg/100 ml, an increase which is statistically significant at the level of $P < 0.005$.

Discussion

Calcium absorption in uremic patients is relatively unaffected by vitamin D administration except when given in high doses. Presumably, these high doses are required to overcome the feedback inhibition of the 25-hydroxylase system [27]. Pertinent to this is the fact that previous studies in this laboratory under conditions virtually identical to those in the present study failed to demonstrate any significant increase in calcium absorption in uremic patients given 5 mg of vitamin D daily for eight days [23]. The administration of 25(OH)D$_3$ bypasses the 25-hydroxylase system and permits the attainment of high circulating concentrations [27]. Evidence accumulated to date suggests that calcium malabsorption in uremia is the result of insufficient production of 1,25(OH)$_2$D$_3$ [28, 29]. However, the administration of 25(OH)D$_3$ in the dosage schedule employed resulted in an increase in calcium absorption even in the absence of renal tissue and presumably, therefore, in the absence of the 25-hydroxycholecalciferol-1-hydroxylase system. This suggests that high circulating concentrations of 25(OH)D$_3$ may be sufficient to stimulate calcium absorption without prior conversion to 1,25(OH)$_2$D$_3$. This conclusion is supported by the recent observation that 1,25(OH)$_2$D$_3$ was not detectable in the serum of anephric patients following the administration of radiolabeled 25(OH)D$_3$ [30] and suggests that 1,25(OH)$_2$D$_3$ cannot be synthesized extrarenally. The possibility of extrarenal conversion of 25(OH)D$_3$ to another or other compounds capable of stimulating calcium absorption is obviously not excluded by this study. Extrarenal production of a compound more polar than 1,25(OH)$_2$D$_3$ has been found [30]. This compound comigrated with authentic 1,24,25(OH)$_3$D$_3$, a compound which has been shown to be capable of stimulating calcium absorption. This polar compound was, however, probably not 1,24,25(OH)$_3$D$_3$ since an enzyme system capable of 1-hydroxylation has been found exclusively in renal tissue in mammals; and this compound was found to be considerably less sensitive to periodate oxidation than 1,24,25(OH)$_3$D$_3$ [30].

An apparent direct effect of 25(OH)D$_3$ is not without precedence since there is evidence that this compound may act directly on bone [31–33]. It is, however, clear from this study that renal tissue is not required for 25(OH)D$_3$ to affect an increase in calcium absorption and to increase the serum calcium concentration in uremic patients; and the possibility exists that this compound may stimulate calcium absorption directly.

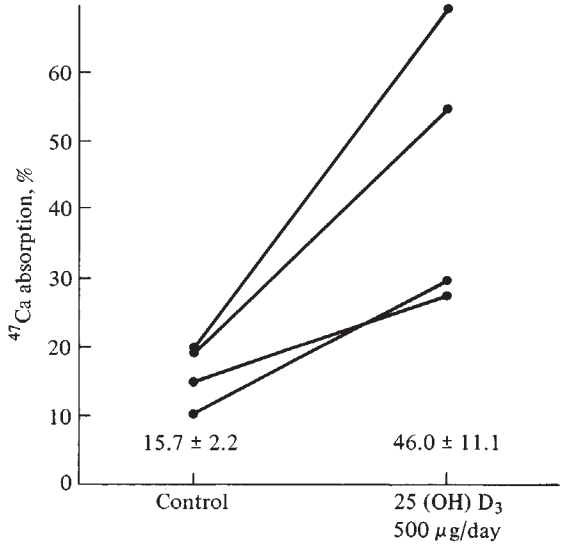

Fig. 3. *Calcium absorption in four anephric patients before and after eight days of treatment with 25(OH)D$_3$, 500 μg daily.*

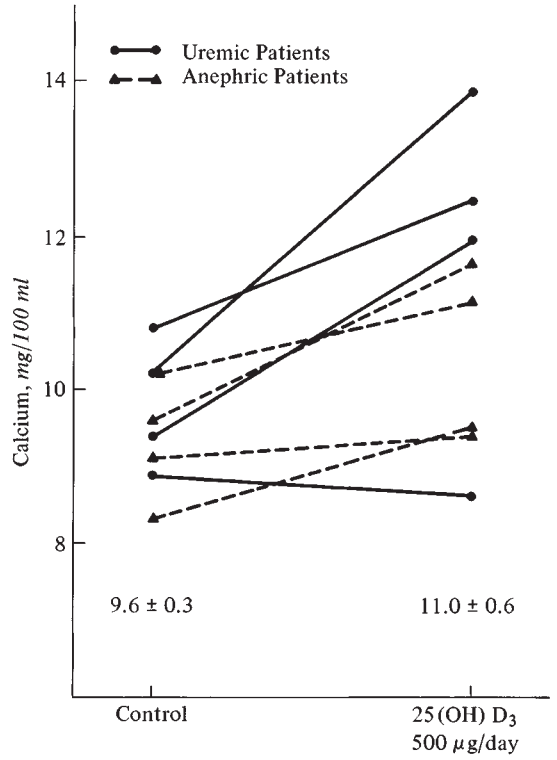

Fig. 4. *Serum calcium for a group of four uremic patients with residual renal function (●—●), and four anephric patients (▲—▲) before and after treatment with 25(OH)D_3, 500 μg daily for eight days.*

The serum calcium concentration increased in most of the patients; however, the increment was independent of renal mass and was not proportional to the increase in calcium absorption. This suggests that bone mineral mobilization may have contributed to the increase in serum calcium concentration. It is possible that the degree and predominant form of renal osteodystrophy, the circulating concentrations of parathyroid hormone as well as other factors, may be important determinants affecting the degree of bone mineral mobilization and thereby affecting the serum calcium concentration independent of the increase in intestinal calcium absorption. Additional studies must be conducted in order to further delineate the factor(s) responsible for the variable rise in serum calcium concentration. It should be emphasized that four patients developed severe hypercalcemia (Fig. 4) after only eight days of treatment, and therefore 500 μg/day of 25 (OH)D_3 appears to be an excessive dose. Furthermore, since all of the factors responsible for the increased calcium concentration are unknown, all patients receiving this drug must be kept under strict medical control and serum calcium concentration must be monitored frequently. We have safely administered smaller doses (50 to 100 μg/day) of 25(OH)D_3 to uremic patients on a long-term basis (*vide infra*).

Observations in this laboratory and the work of others suggest that 25(OH)D_3 may also be beneficial in the treatment of secondary hyperparathyroidism and renal osteodystrophy. The nature of this study did not allow us to gain useful information regarding changes in parathyroid hormone concentrations in blood or correction of osteomalacia. However, findings of long-term studies in uremic dogs receiving 25(OH)D_3 indicate a complete prevention of secondary hyperparathyroidism in these animals [34]. Other investigators [35, 36] demonstrated that 25(OH)D_3 was active on bone of uremic patients. We also have found (Teitelbaum S, Kopelman R, Hruska K, Rutherford WE, Slatopolsky E: Unpublished observations) improvement in both the osteitis fibrosa and osteomalacia in patients with advanced renal failure.

The present study indicates that 25(OH)D_3 may be a practical therapeutic adjunct in the treatment of calcium malabsorption in uremic patients even in the absence of the 1-hydroxylase system. The ultimate utility of 25(OH)D_3 as well as that of other metabolites and/or analogues of vitamin D will, however, depend on the economy, ease and safety with which they may be employed to affect overall homeostatic calcium balance.

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