Changes in plasma half-life and clearance of ^3H -25-hydroxyvitamin D_3 in patients with intestinal malabsorption

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SUMMARY The plasma disappearance of intravenously administered tracer doses of tritium-labelled 25-hydroxyvitamin D_3 (25OHD_3) was studied in six normal subjects and in 15 patients with intestinal malabsorption. The plasma half-life was significantly shorter and the clearance rate significantly greater in the group with malabsorption compared with the controls. One explanation for this increased elimination could be interruption of an enterohepatic circulation of 25OHD occurring in subjects with malabsorption and such a mechanism could account for the loss of endogenous vitamin D in these patients.

Vitamin D deficiency and osteomalacia occur in a variety of intestinal disorders¹⁻³ and are common after jejunoileal bypass (JIB) for obesity.³⁻⁵ As the major source of vitamin D in man is from endogenous synthesis in the skin,⁶ malabsorption of dietary vitamin D may not be an important cause of vitamin D deficiency in these patients, providing that exposure to ultraviolet irradiation is normal. If, however, as has been suggested,⁷ 25-hydroxyvitamin D (25OHD) undergoes enterohepatic circulation in man, interruption of this circulation by intestinal disease, resection, or bypass could lead to increased loss of both exogenous and endogenous vitamin D. Increased faecal loss of endogenously derived 25OHD_3 should be associated with a reduced plasma half-life and increased clearance of the metabolite; in support of this, Arnaud *et al*⁸ reported a reduced plasma half-life of ^3H - 25OHD_3 after its intravenous administration in four patients with coeliac disease. We have studied the plasma disappearance of radiolabelled tracer doses of 25OHD_3 in six normal adults and in 15 patients with intestinal malabsorption.

Methods

PATIENTS

The control group consisted of six healthy males aged 28-33 years (mean 30 years). Fifteen patients, nine females and six males, aged 24-66 years (mean 50 years) were studied. Eight had undergone small intestinal resection, for Crohn's disease in six, carcinoid in one, and abdominal trauma in one; of these, six had also had some colonic resection. The length of remaining small intestine ranged from 60-270 cm (mean 126 cm). Four patients had undergone end-to-side anastomosis of 18 cm of proximal jejunum to 18 cm of terminal ileum as treatment for gross obesity; in one of these, a cholecystojejunostomy was performed two years before the study. Two patients had coeliac disease and one had unoperated Crohn's disease with multiple small intestinal strictures. All patients had normal renal and hepatic function as assessed by plasma creatinine and conventional liver function tests. Four patients were receiving steroid therapy (prednisone or synacthen), three were receiving steroid and vitamin D therapy, two vitamin D only, and one vitamin D and phenytoin. Vitamin D therapy consisted of 150,000-300,000 units intramuscularly monthly in three patients and oral 1α -hydroxyvitamin D_3 ($1\alpha\text{OHD}_3$), 2 μg daily, in the remaining three. All patients gave informed consent

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to the study, which was approved by the hospital Ethical Committee. The study was carried out between October 1980 and August 1981.

25-hydroxy-(23,24(n)- ^3H)vitamin D_3 (120 Ci/mmol) or 25-hydroxy-(26(27)methyl- ^3H)vitamin D_3 (9.6 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, Bucks. 2–5 μCi in 15% ethanol was injected intravenously over three to five minutes. Blood samples were taken at 10 minutes, hourly for six hours and at intervals of one to three days for 30 days. Plasma radioactivity was measured by direct scintillation counting in Nuclear Enterprises 260 scintillation fluid, using an LKB Rackbeta liquid scintillation counter. Quench correction was applied using the channels ratio method.

Plasma 25OHD concentrations were measured by a competitive protein-binding assay⁹ using normal human serum as binding protein. The proportion of plasma radioactivity eluting as ^3H -25OHD $_3$ after injection was measured by chloroform/methanol extraction and silicic acid chromatography on weekly plasma samples from the control subjects.

The measurements of plasma radioactivity were used to construct log plasma concentration/time curves for each subject and the gradient of the slope from the fourth day onwards was determined by least squares regression analysis. The relationship half-life ($T_{1/2}$) = $\log_{2e}/\text{gradient}$ was used to calculate the plasma half-life for each subject. Clearance rates were derived from the dose given and the area under the plasma concentration/time curve (AUC) according to the formula:

$$\text{clearance} = \frac{\text{Slope} \times \text{dose}}{\text{AUC}}$$

Differences between the control and study groups were assessed using Student's unpaired t test.

Results

The log plasma concentration/time curves demonstrated an early rapid exponential phase lasting about four hours. After three to four days, a second much slower exponential was seen, lasting for at least 30 days. The gradient of the second exponential was considered to represent elimination of the isotope after the attainment of distribution equilibrium.

The mean plasma half-life of ^3H -25OHD $_3$ in the controls was 27.5 ± 2.1 days ($\pm \text{SEM}$), compared with 14.2 ± 1.4 days in the patients with intestinal malabsorption ($p < 0.001$) (Fig. 1). The mean ^3H -25OHD $_3$ clearance in the control group was 3.4 ± 0.3 ml/kg/day, which was significantly lower than in the patient group (6.5 ± 0.7 ml/kg/day; $p < 0.02$) (Fig. 2).

Plasma 25OHD concentrations in the control group were all normal (mean 80 nmol/l; normal

Fig. 1 Plasma half-life of ^3H -25-hydroxyvitamin D_3 in the normal subjects and in the patients with malabsorption. Mean values for each group are indicated by the continuous horizontal line. ○ = subject in whom a cholecystojejunostomy was performed after jejunoileal bypass.

range 12–100 nmol/l). Five patients had 25OHD levels below 12 nmol/l; of these, three were receiving $1\alpha\text{OHD}_3$ therapy. The remaining nine patients had normal plasma 25OHD levels (mean 62.5 nmol/l), including all three patients who were receiving parenteral vitamin D_2 . There was no significant correlation between the plasma 25OHD level and the plasma ^3H -25OHD $_3$ half-life or clearance in either group.

Extraction and chromatography of plasma samples from the control group demonstrated that at least 90% of the radioactivity eluted as ^3H -25OHD $_3$ at 24 hours and at least 80% at 30 days after injection of either isotope of 25OHD $_3$. In patients with malabsorption the corresponding figures were 84% and 75%.

Fig. 2 Plasma clearance of ^3H -25-hydroxyvitamin D_3 in the normal subjects and in the patients with malabsorption. Mean values for each group are shown by the continuous horizontal line. The symbol $\circ$ is used as in Fig. 1.

Discussion

Our results demonstrate that in patients with intestinal malabsorption the plasma half-life of intravenously administered ^3H -25OHD $_3$ is significantly shorter and the clearance rate significantly greater than in normal subjects. These findings indicate that loss of endogenous 25OHD $_3$ may be increased in malabsorption; although we did not demonstrate increased faecal loss of ^3H -25OHD $_3$ in these experiments, our previous finding of increased faecal tritium excretion after intravenous ^3H -25OHD $_3$ in patients with malabsorption¹⁰ suggests that the reduction in plasma half-life and increased clearance of ^3H -25OHD $_3$ may be due to increased loss from an enterohepatic circulation of the

metabolite. The relatively normal plasma half-life and clearance of ^3H -25OHD $_3$ in the patient with a cholecystojejunostomy would be consistent with this hypothesis, as enterohepatic circulations of bile acids and 25OHD should be preserved after this operation.

25OHD is the major circulating form of vitamin D in man and provides a pool for the synthesis of biologically active metabolites. Biliary excretion of metabolites of ^3H -25OHD $_3$ has been demonstrated in man after its intravenous administration, although quantitative estimates of the amount of radioactivity excreted vary from 12–35% of the injected dose.^{7–11} Most of the biliary radioactivity is in the form of biologically inactive water soluble conjugates, but the form in which radioactivity is reabsorbed is unknown and direct evidence for a conservatory enterohepatic circulation in man is lacking at present.

There is evidence that the turnover of vitamin D and its metabolites is affected by vitamin D status, being more rapid in vitamin D deplete subjects;¹² thus it is possible that vitamin D deficiency may have contributed to the shorter plasma half-life of ^3H -25OHD $_3$ in some of our patients. Using plasma 25OHD as an indicator of vitamin D status, however, only one-third of our patients were vitamin D deficient and, of these five, three were receiving oral 1 α OHD $_3$ therapy and may therefore not have been strictly vitamin D deficient. In addition, there was no significant correlation between the initial plasma 25OHD concentration and either plasma half-life or clearance of ^3H -25OHD $_3$. Nevertheless, we cannot exclude the possibility that the shorter plasma half-life in patients with malabsorption was related to vitamin D depletion.

Another factor which might affect our results is the steroid therapy taken by seven patients. The effects of corticosteroids on vitamin D metabolism are disputed and low,¹³ normal,¹⁴ or high¹⁵ plasma 25OHD concentrations have been reported in corticosteroid treated patients. The finding of impaired conversion of vitamin D to 25OHD in patients receiving large doses of corticosteroids¹⁶ was not confirmed in corticosteroid treated rats.¹⁷ The effects of corticosteroids on plasma half-life and clearance of ^3H -25OHD $_3$ in man are not reported and we cannot exclude the possibility that steroid therapy contributed to the enhanced elimination of ^3H -25OHD $_3$ in some patients. Similarly, phenytoin therapy in one patient may have affected the plasma disappearance of the metabolite, although there are no reports of phenytoin therapy alone reducing the plasma half-life of ^3H -25OHD $_3$.

Although the administration of ^3H -25OHD $_3$ as an

intravenous bolus is unphysiological, the plasma half-life and clearance rate in this study were calculated from the plasma disappearance of the isotope after distribution equilibrium had been attained and thus our results should reflect the behaviour of the natural metabolite. It is possible that increased loss of ^3H -25OHD $_3$ through the intestinal mucosa contributed to the increased plasma elimination of the metabolite in patients with intestinal disease. Reduced intraduodenal bile acid concentrations due to interruption of the enterohepatic circulation of bile acids might also contribute, although there is evidence in rats and in man that the absorption of 25OHD is not bile acid dependent.^{18 19}

In conclusion, we have demonstrated significantly increased plasma elimination of intravenous ^3H -25OHD $_3$ in patients with intestinal malabsorption. Although we have not defined the mechanism of this increased loss, it could be explained by interruption of an enterohepatic circulation of 25OHD. Whatever the mechanism of the reduced plasma half-life and increased clearance of ^3H -25OHD $_3$ in our patients, our findings indicate that loss of endogenous 25OHD $_3$ is increased in malabsorption and provide an explanation for the development of vitamin D deficiency and osteomalacia in patients with normal endogenous vitamin D_3 synthesis who have intestinal disease, resection, or bypass.

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References

- Compston JE, Creamer B. Plasma levels and intestinal absorption of 25-hydroxyvitamin D_3 in patients with small bowel resection. *Gut* 1977; **18**: 171-5.
- Driscoll RH, Meredith S, Wagonfeld JW, Rosenberg IH. Bone histology and vitamin D status in Crohn's disease. *Gastroenterology* 1977; **72**: 1051.
- Schoen MS, Lindenbaum J, Roginsky MS, Holt PR. Significance of serum level of 25-hydroxyvitamin D in gastrointestinal disease. *Dig Dis Sci* 1978; **23**: 137-42.
- Teitelbaum SL, Halverson JD, Bates M, Wise L, Haddad JG. Abnormalities of circulating 25-hydroxyvitamin D after jejunio-ileal bypass for obesity. *Ann Intern Med* 1977; **86**: 289-93.
- Compston JE, Laker MF, Woodhead JS *et al.* Bone disease after jejunio-ileal bypass for obesity. *Lancet* 1978; **2**: 1-4.
- Haddad JG, Hahn TJ. Natural and synthetic sources of circulating 25-hydroxyvitamin D in man. *Nature* 1973; **244**: 515-7.
- Arnaud SB, Goldsmith RS, Lambert PW, Go VLW. 25-hydroxyvitamin D_3 : evidence of an enterohepatic circulation in man. *Proc Soc Exp Biol Med* 1975; **149**: 570-2.
- Arnaud SB, Newcomer AD, Offord KP *et al.* 25-hydroxyvitamin D (25OHD) metabolism in non-tropical sprue (NTS). In: Norman AW, Schaefer K, Herrath DV *et al.*, eds. *Vitamin D, basic research and its clinical application*. Berlin, New York: Walter de Gruyter, 1979: 1023-6.
- Edelstein S, Charman M, Lawson DEM, Kodicek E. Competitive protein-binding assay for 25-hydroxycholecalciferol. *Clin Sci* 1974; **46**: 231-40.
- Compston JE, Merrett AL, Ledger JE, Creamer B. Faecal tritium excretion after intravenous administration of ^3H -25-hydroxyvitamin D_3 in control subjects and in patients with malabsorption. *Gut* 1982; **23**: 310-15.
- Gray RW, Weber HP, Dominguez JH, Lemann J. The metabolism of vitamin D_3 and 25-hydroxyvitamin D_3 in normal and anephric humans. *J Clin Endocrinol Metab* 1974; **39**: 1045-56.
- Mawer EB, Lumb GA, Schaefer K, Stanbury SW. The metabolism of isotopically labelled vitamin D_3 in man: the influence of the state of vitamin D nutrition. *Clin Sci* 1971; **40**: 39-53.
- Klein RG, Arnaud SB, Gallagher JC, De Luca HF, Riggs BL. Intestinal calcium absorption in exogenous hypercortisolemia. *J Clin Invest* 1977; **60**: 253-9.
- Hahn TJ, Halstead LR, Haddad JG. Serum 25-hydroxyvitamin D concentrations in patients receiving chronic corticosteroid therapy. *J Lab Clin Med* 1977; **90**: 399-404.
- Lukert BP, Adams JS. Vitamin D metabolism in man. Effect of corticosteroids. *Arch Intern Med* 1976; **136**: 1241-8.
- Avioli LV, Birge SJ, Lee SW. Effects of prednisone on vitamin D metabolism in man. *J Clin Endocrinol Metab* 1968; **28**: 1341-6.
- Kimberg DV, Baerg RD, Gershon E, Granducius RT. Effect of cortisone treatment on the active transport of calcium by the small intestine. *J Clin Invest* 1971; **50**: 1309-21.
- Nechama H, Hoff D, Harell A, Edelstein S. The intestinal absorption of vitamin D and its metabolites. *J Mol Med* 1977; **2**: 413-22.
- Compston JE, Merrett AL, Hammett FG, Magill P. Comparison of the appearance of radiolabelled vitamin D_3 and 25-hydroxyvitamin D_3 in the chylomicron fraction of plasma after oral administration in man. *Clin Sci* 1981; **60**: 241-3.