

PART XLIII

CALCIUM – VITAMIN D – PARATHYROID HORMONES

Chairmen: A Fournier
R Verberckmoes

EXTRA-RENAL PRODUCTION OF 1,25(OH)₂D₃ AND 24,25(OH)₂D₃ IN ANEPHRIC PATIENTS

G Graziani, *S Adami, S Casati, A Cantaluppi, *D Tartarotti, C Castelnovo, *V Lo Cascio, C Ponticelli

*Ospedale Maggiore, Milan, *Istituto di Semeiotica Medica, Università di Verona, Verona, Italy*

Summary

To evaluate the existence of extra-renal 1 α -hydroxylase and 24-hydroxylase activity in organs other than kidney, 150 μ g of 25(OH)D₃ were administered daily for 15 days to seven anephric patients. None of them had received vitamin D₃ or derivatives in the previous six months, or vitamin D₂ in the last five years. Plasma levels of 25(OH)D₃, 1,25(OH)₂D₃ and 24,25(OH)₂D₃ were measured before and at withdrawal of therapy. Patients compliance to 25(OH)D₃ therapy was confirmed by the significant increment of plasma levels of the hormone. Pre-treatment plasma levels of 24,25(OH)₂D₃ were 0.29 \pm 0.25ng/ml and increased to 0.99 \pm 0.67ng/ml after therapy (p<0.02). Baseline plasma levels of 1,25(OH)₂D₃ increased from 6.1 \pm 3.6pg/ml to 11.7 \pm 3.9pg/ml (p<0.02) at withdrawal. Our results indicate an extra-renal, substrate dependent, activity of 1 α - and 24-hydroxylases.

Introduction

It is generally agreed that the kidney is the sole site of synthesis of 1 α - and 24-hydroxylated metabolites of vitamin D. Recently the existence of sites of synthesis of these active metabolites other than the kidney has been described [1-4].

The aim of this study was to confirm the existence of extra-renal 1 α - and 24-hydroxylase synthesis and to evaluate the possibility of enhancing their production by exogenous administration of 25(OH)D₃ to anephric patients.

Patients and methods

Seven anephric patients (2 women and 5 men; mean age 47 \pm 14 years) on regular dialysis treatment for 12 \pm 5 years were studied. These patients had neither received vitamin D₃ nor its derivatives in the six months preceding the study

nor taken vitamin D₂ during the last five years. Plasma levels of total and ionized calcium, phosphate, alkaline phosphatase, 25(OH)D₃, 24,25(OH)₂D₃ and 1,25(OH)₂D₃ were measured before and after 15 days of daily administration of 150µg of 25(OH)D₃. The study was conducted in February and March. Plasma concentrations of total calcium, ionized calcium and alkaline phosphatase were measured by automated methods. Vitamin D₃ metabolites were measured by radioassay after extraction of serum with C-18 Sep-paks and fractionation by high pressure liquid chromatography on Zorbax-SIL eluted with a ternary solvent system of hexane: methanol: isopropanol (92:4:4 by volume) at a flow rate of 2.0ml/min [5]. Concentrations of 25(OH)D₃ were measured by a competitive binding assay [6]. The limits of detection of these assays were 0.5ng/ml for 25(OH)D₃, 0.2ng/ml for 24,25(OH)₂D₃ and 4.0ng/ml for 1,25(OH)₂D₃. Data were analysed by the Student's 't' test for paired data.

Results

No patient had any untoward effect to 25(OH)D₃ administration. Serum 25(OH)D₃ was below the normal range in five of seven patients; serum 1,25 and 24,25(OH)₂D₃ were below normal in all patients and untitratable in three of the seven. As shown in Table I, after the 25(OH)D₃ loading period, no significant variations of total and ionized calcium, phosphate or alkaline phosphatase were observed, whereas there were significant increases in plasma levels of 25(OH)D₃, 24,25(OH)₂D₃ and 1,25(OH)₂D₃.

TABLE I. Plasma levels of different biochemical values in dialysis patients before and after 25(OH)D₃ treatment

| | Normal range | Before therapy | After therapy | p |
|---|--------------|----------------|---------------|--------|
| Total Ca (mg/dl) | 8.5–10.5 | 9.7±0.98 | 9.97±0.36 | NS |
| Ionized Ca (mg/dl) | 4.1–5.2 | 4.5±0.42 | 4.30±1.0 | NS |
| Phosphate (mg/dl) | 3.0–4.5 | 4.1±1.12 | 4.25±1.25 | NS |
| Alkaline phosphatase (U/L) | 67–172 | 376±257 | 412±281 | NS |
| 25(OH)D ₃ (ng/ml) | 5–40 | 5.78±5.0 | 64.7±31.6 | <0.001 |
| 1,25(OH) ₂ D ₃ (pg/ml) | 20–70 | 6.1±3.6 | 11.7±3.9 | <0.02 |
| 24,25(OH) ₂ D ₃ (ng/ml) | 0.3–3.0 | 0.29±0.25 | 0.99±0.67 | <0.02 |

Data as mean ± SD

p indicates significance between pre- and post-treatment values

Discussion

In these patients serum 25(OH)D₃ was found to be frequently below normal during the winter season in which this study was conducted. This suggests that in Northern Italy, where the dairy products do not contain vitamin D supplements, 25(OH)D₃ levels of uraemic patients should be periodically checked.

Detectable concentrations of $24,25(\text{OH})_2\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ were found in the patients with plasma levels of $25(\text{OH})\text{D}_3$ greater than 4.6ng/ml after therapy. After $25(\text{OH})\text{D}_3$ administration, serum $25(\text{OH})\text{D}_3$ levels rose and this confirms the compliance of the patients in taking the vitamin D therapy. $24,25(\text{OH})_2\text{D}_3$ became detectable in all patients and it reached normal values in six of seven patients. Serum $1,25(\text{OH})_2\text{D}_3$ became detectable in all patients but remained below the normal range in all cases. This suggests that there is some extra-renal substrate-dependent synthetic activity of both 1α - and 24-hydroxylases. Extra-renal synthesis of $1,25(\text{OH})_2\text{D}_3$ has been demonstrated in cultured human bone cells [7] and decidua [8]. Other investigators have confirmed that natural and synthetic vitamin D preparations stimulate the production of $24,25(\text{OH})_2\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ in anephric patients [1–3]. However, many of those patients had been treated with dihydrotachysterol, which might have caused spuriously high $1,25(\text{OH})_2\text{D}_3$ plasma levels. In this study we have shown that plasma levels of $25(\text{OH})\text{D}_3$ greater than 40ng/ml are usually required to obtain normal levels of $24,25(\text{OH})_2\text{D}_3$. The role of $24,25(\text{OH})_2\text{D}_3$ in the treatment of renal osteodystrophy still remains controversial, but therapy with $25(\text{OH})\text{D}_3$ acutely increases the plasma levels of $24,25(\text{OH})_2\text{D}_3$ a metabolite not yet available commercially. Serum $1,25(\text{OH})_2\text{D}_3$ was detectable but below normal despite supraphysiological concentrations of $25(\text{OH})\text{D}_3$. This implies that if normal plasma concentrations of $1,25(\text{OH})_2\text{D}_3$ are desired this metabolite must be administered per se.

References

- 1 Lambert PW, Stern PH, Avioli RC et al. *J Clin Invest* 1982; 69: 722
- 2 Zerwekh JE, McPhaul JJ, Parker TF, Pak CYC. *Kidney Int* 1983; 23: 401
- 3 Jongen MJM, van der Vijgh WJF, Lips P, Netelenbos JC. *Nephron* 1984; 36: 230
- 4 Rickers H, Christiansen C, Christiansen P et al. *Nephron* 1985; 39: 267
- 5 Tartarotti D, Adami S, Galvanini G et al. *J Endocrinol Invest* 1984; 7: 545
- 6 Preece MA, O'Riordan JLH, Lawson DEM, Kodicek E. *Clin Chim Acta* 1974; 54: 235
- 7 Howard GA, Turner RT, Sherrard DJ, Baylink DJ. *J Biol Chem* 1981; 256: 7738
- 8 Weisman YA, Harrell S, Edelstein M et al. *Nature (Lond)* 1978; 281: 317