Vitamin D metabolites in idiopathic infantile hypercalcaemia

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SUMMARY Metabolites of vitamin D were measured in plasma from 83 patients with idiopathic infantile hypercalcaemia syndrome who were mentally handicapped but had normal calcium values at the time of the study. No significant difference was detected in the mean plasma concentrations of 25-hydroxyvitamin D₂, 1,25-dihydroxyvitamin D, 24,25-dihydroxyvitamin D₃, or 25,26-dihydroxyvitamin D₃ between patients and age matched controls. The mean plasma concentration of 25-hydroxyvitamin D₃ was significantly lower in patients than controls but this may be a secondary phenomenon related to less sunlight exposure. In addition, two hypercalcaemic patients with this syndrome were studied during the first year of life, and were found to have normal concentrations of vitamin D metabolites. These findings do not support a role for abnormal vitamin D metabolism in the pathogenesis of this syndrome.

Idiopathic infantile hypercalcaemia may be associated with a congenital dysmorphic syndrome which is characterised by the elfin facies, supra-aortic or pulmonary stenosis, mental handicap, and dental abnormalities.¹⁻³ We have recently reviewed 117 patients with this dysmorphic syndrome, 76 of whom had documented evidence of hypercalcaemia during infancy, highlighting some of the clinical features.⁴

The cause of this syndrome is unknown but Lightwood⁵ was the first to suggest that an abnormality of vitamin D metabolism might cause the hypercalcaemia. Calcium balance studies showed increased intestinal absorption^{6,7} during the acute illness, but a history of excessive vitamin D intake is usually lacking, hence the hypothesis that these infants are 'vitamin D sensitive'.⁵

Only three previous studies have measured the plasma metabolites of vitamin D in this syndrome. Aarskog *et al*⁸ measured 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in one patient during the hypercalcaemic phase, and the concentration of each metabolite was normal. (Throughout our paper, unless it is specifically stated, any measurement of a metabolite of 'vitamin D' implies that the total metabolites of vitamin D₂ and D₃ have been estimated.) Taylor *et al*⁹ gave a pharmacological dose of vitamin D₂ (1500 IU/kg) to six patients with normal calcium values whose mean age was 8 years,

and reported normal baseline concentration of 25-hydroxyvitamin D but a greater increase in 25-hydroxyvitamin D concentrations in cases than age matched controls. More recently, Garabedian *et al*¹⁰ described raised 1,25-dihydroxyvitamin D concentrations that seemed to decrease over the first 2 to 4 years of life, in four patients with this syndrome. Three of these patients, however, were not mentally handicapped, had no cardiovascular abnormality, and were diagnosed purely on the presence of an elfin facies. The only infant with hypercalcaemia, mental handicap, and a cardiovascular anomaly had normal 1,25-dihydroxyvitamin D values.

We have previously reported a clinical study of 117 patients with this syndrome, and report here the plasma concentrations of vitamin D metabolites in a random sample of 83 of these, an age matched control group, and two further patients with the same syndrome during the hypercalcaemic phase of their illness. Specific high performance liquid chromatographic and mass fragmentographic assays were used.

Patients

All patients fulfilled the selection criteria suggested by Martin *et al*.⁴ Their sex ratio and mean age are shown in Table 1. They were divided into two groups depending on the presence (group 1) or

Table 1 Details of each study group. Group 1 comprised patients with, and group 2 those without, documented hypercalcaemia.

	Group 1	Group 2	Controls
No	52	31	33
Age (yrs). Mean (SEM)	11.8 (1.1)	13.0 (1.4)	11.6 (1.9)
Boy:Girl	30:22	16:15	21:12
Sample date.* Mean (SEM)	0.36 (0.03)	0.29 (0.04)	0.36 (0.03)

*Blood sample date is expressed as the decimal of a year and the mean date was calculated for each group.

absence (group 2) of documented hypercalcaemia in infancy. A control group was drawn from caucasian children admitted to hospital for routine elective surgery, with no clinical suspicion of an abnormality of calcium metabolism, or from healthy members of the laboratory staff.

Two children were studied soon after diagnosis at 6 months (case 1) and 11 months (case 2) of age, before treatment of their hypercalcaemia. Both were developmentally delayed, had elfin facies, and a cardiac murmur. Case 1 had nephrocalcinosis and case 2 had echocardiographic evidence of mild supravulvular aortic stenosis. In both, calcium values became normal on treatment with a low calcium diet, and on follow up, both conform to the clinical syndrome.

Methods

Blood samples were taken at varying times throughout one year, the day of sampling is expressed as a decimal of one year and the mean was calculated for each group (Table 1). Plasma was separated immediately and stored at -20°C until analysed. Metabolites of vitamin D were extracted from plasma as described by Rhodes *et al*¹¹ using commercially available, prepacked microparticulate silica cartridges (Sep-Pak C18; Waters Associates, USA). Subsequent fractionation on Sep-Pak Sil cartridges gave three fractions, vitamin D, 25-hydroxyvitamin D, and polyhydroxylated vitamin D. Concentrations of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 were assayed by high performance liquid chromatography after isomerisation to isotachysterol.¹² Concentrations of 24,25-dihydroxyvitamin D3 and 25,26-dihydroxyvitamin D3 were assayed by gas chromatography mass fragmentography.^{13 14} The 1,25-dihydroxyvitamin D value was estimated by receptor binding assay using the 1,25-dihydroxyvitamin D receptor protein obtained from chick duodenum cytosol.¹⁵ This assay does not distinguish between 1,25-dihydroxyvitamin D2 and 1,25-dihydroxyvitamin D3. Statistical comparisons were made with the Mann-Whitney U test (two tailed).

Results

The mean (SEM) values of each metabolite in each of the study groups are shown in Table 2 and the values obtained for the two hypercalcaemic infants in Table 3.

There were no significant differences in the concentrations of any metabolite between groups 1 and 2 but the plasma 25-hydroxyvitamin D3 concentration in each of the patient groups was significantly less than in controls. Plasma 25,26-dihydroxyvitamin D3 was also significantly lower in both patient groups compared with controls, and there was a similar trend, just failing to reach conventional significance, for 24,25-dihydroxyvitamin D3. There was no significant difference between any of the groups in the case of 25-hydroxyvitamin D2 or 1,25-dihydroxyvitamin D. Plasma values of 24,25-dihydroxyvitamin D3 and 25,26-dihydroxyvitamin D3 correlated significantly ($P<0.05$) with 25-hydroxyvitamin D3 values ($r=0.67, 0.57$ respectively).

The two hypercalcaemic patients have been considered separately; these patients were studied

Table 2 The mean (SEM) plasma concentrations of the metabolites of vitamin D2 and vitamin D3 in each study group. Samples were not always sufficient for each estimation and the number assayed in each group are shown in parenthesis below the mean (SEM) values

	Group 1	Group 2	Group 3
25OHD2 (ng/ml)	3.3 (0.7)	2.0 (0.3)	2.6 (0.6)
(n)	(46)	(27)	(27)
25OHD3 (ng/ml)	14.7 (0.9)*	14.0 (1.4)*	21.0 (2.4)
(n)	(52)	(30)	(30)
24,25OHD3 (ng/ml)	0.75 (0.10)†	0.84 (0.14)	1.14 (0.24)
(n)	(40)	(22)	(20)
25,26OHD3 (ng/ml)	0.19 (0.02)*	0.20 (0.03)*	0.35 (0.07)
(n)	(31)	(19)	(10)
1,25OHD (pg/ml)	51.7 (7.1)	53.7 (17.7)	58.5 (12.2)
(n)	(38)	(20)	(17)

*Groups 1 and 2 v controls $P<0.05$.

†Groups 1 and 2 v controls $P=0.1$.

OHD=dihydroxyvitamin D.

Table 3 Biochemical data on the two infants with hypercalcaemia and idiopathic infantile hypercalcaemia. The reference range for each vitamin D metabolite is also given and was derived from the control group (Table 2) whose mean age was 11.6 years, ranging from 1–28 years

	Case 1	Case 2	Controls Mean (SD)
Age (mths)	6	11	—
Plasma calcium (mmol/l)	3.4	3.2	—
25OHD2 (ng/ml)	33.1	8.0	2.6 (2.9)
25OHD3 (ng/ml)	15.3	26.1	21.0 (13.1)
24,25OHD3 (ng/ml)	1.06	3.42	1.1 (1.1)
1,25OHD (pg/ml)	28.6	43.5	58.5 (49.1)

independently and very similar results were obtained (McGraw M, Mawer E B, personal communication).

Discussion

All but two patients in this study had normal calcium values, but as recurrence of hypercalcaemia has been reported in at least one case,⁴ it was theoretically possible that changes in the vitamin D metabolites might be detected in the 'burnt out phase' of the syndrome. Significantly lower concentrations of 25-hydroxyvitamin D₃ have been found in patients with this syndrome, but this is unlikely to be of aetiological importance. It seems more probable that this is a secondary phenomenon related to their mental handicap since these subjects may have a less active, more sedentary, indoor lifestyle with less exposure to sunlight.

Although no mentally handicapped control group was available to confirm this hypothesis, Taylor *et al*⁹ studied six patients with this syndrome. Their data showed the same trend, although their assay measured only total 25-hydroxyvitamin D, and did not distinguish between the individual 25-hydroxy derivatives of vitamin D₂ and D₃. Moreover, these lower substrate values may well explain their findings of increased 25-hydroxyvitamin D synthesis after pharmacological challenge with vitamin D₂, since there is evidence for the induction of 25-hydroxylase activity in states of relative substrate deficiency.¹⁶

The two hypercalcaemic infants we studied also failed to show any abnormality in the metabolism of vitamin D. The concentrations of the active vitamin D hormone responsible for calcium absorption from the gut, 1,25-dihydroxyvitamin D, were within the reference range for our control group, whose mean age was 11.6 years, and indeed tended to be at the lower end of this range. Lund *et al*¹⁷ have shown higher concentrations of 1,25-dihydroxyvitamin D in the first two years of life than in later childhood, and a significant positive correlation with growth velocity. Therefore the low 1,25-dihydroxyvitamin D values found in these two patients suggests that there is either appropriate suppression of 1 α -hydroxylase activity in the presence of hypercalcaemia or perhaps that their growth velocity is reduced secondary to their hypercalcaemia.

The 25-hydroxyvitamin D₂ values seem, at first sight, to be raised, since the ratio of 25-hydroxyvitamin D₃:25-hydroxyvitamin D₂ for adults is in the order of 9:1. For infants fed 'fortified' milk feeds, however, 25-hydroxyvitamin D₂ values are certainly higher, and have been shown to rise during

the first month of life.¹⁸ Certainly they are not within the range associated with vitamin D toxicity.

Although confirmation of these findings in other infants with idiopathic infantile hypercalcaemia is required, this is the first comprehensive study of vitamin D metabolites in a large group of patients with well documented idiopathic infantile hypercalcaemia. No specific abnormality of vitamin D metabolism in either the hypercalcaemic or normocalcaemic phases of this syndrome has been identified, and we have also failed to detect any abnormality of the kinetics of 25-hydroxyvitamin D₃ turnover in adolescents with this syndrome who are in the normocalcaemic phase.

Thus, although abnormalities in the metabolism of both 25-hydroxyvitamin D⁹ and 1,25-dihydroxyvitamin D¹⁰ have been suggested as the cause of hypercalcaemia in this syndrome, we have been unable to show any abnormality of vitamin D metabolism. This is in agreement with the findings of Aarskog *et al*.⁸ We suggest that more patients with clear evidence of idiopathic infantile hypercalcaemia and the associated dysmorphic syndrome, which is perhaps best described by its eponym The Williams-Beuren syndrome, require to be studied during and after the hypercalcaemic phase, using specific high performance chromatographic and mass fragmentographic assays to estimate the individual metabolites of vitamin D₂ and vitamin D₃. Further study is also needed to examine the relation between hypercalcaemia and mental handicap and the characteristic cardiovascular anomalies.

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