Treatment of osteomalacia associated with primary biliary cirrhosis with parenteral vitamin D₂ or oral 25-hydroxyvitamin D₃

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SUMMARY The histological and biochemical response of osteomalacia has been studied in four patients with primary biliary cirrhosis, who were treated with oral 25-hydroxyvitamin D₃, 50 µg daily, or intramuscular vitamin D₂, 150 000 units once weekly, for five to 12 months. All patients showed complete histological healing of osteomalacia, despite rapidly deteriorating liver function in three. Plasma 25-hydroxyvitamin D concentrations were low in all patients before treatment, but became normal during either vitamin therapy. Serum calcium and phosphate levels, and urinary calcium excretion were not always reliable in predicting the histological response to treatment. Serum alkaline phosphatase activity decreased in all patients during vitamin D therapy. We conclude that both high-dose parenteral vitamin D₂ and oral 25-hydroxyvitamin D₃ may be effective in healing osteomalacia associated with primary biliary cirrhosis. Measurement of plasma 25-hydroxyvitamin D levels during vitamin D therapy provides useful information about 25-hydroxylation of the parent vitamin and intestinal absorption of orally administered 25-hydroxyvitamin D₃.

Vitamin D is 25-hydroxylated in the liver to 25-hydroxyvitamin D (25-OHD) (Ponchon *et al.*, 1969), which is further hydroxylated in the kidney (Fraser and Kodicek, 1970) to 1,25-dihydroxyvitamin D (1,25-OH)₂D (Holick *et al.*, 1971), the major active metabolite. Plasma concentrations of 25-OHD are reduced in most patients with primary biliary cirrhosis (PBC) (Long *et al.*, 1976; Wagonfeld *et al.*, 1976); possible causes include malabsorption of dietary vitamin D (Thompson *et al.*, 1966; Krawitt *et al.*, 1977), and of 25-OHD excreted into the bile and undergoing enterohepatic circulation (Arnaud *et al.*, 1975; Compston and Thompson, 1977), and increased urinary loss of vitamin D (Krawitt *et al.*, 1977). Hepatic 25-hydroxylation of vitamin D may also be impaired in PBC (Wagonfeld *et al.*, 1976), although most patients achieve normal plasma 25-OHD concentrations if given sufficient precursor vitamin D (Skinner *et al.*, 1977).

Osteomalacia develops in some patients with PBC (Compston and Thompson, 1977; Long *et al.*,

1978a) and large parenteral doses of vitamin D are usually given for its prophylaxis or treatment. However, very few studies of bone histology have been carried out after treatment, and the therapeutic dose range has not been strictly defined, nor is it certain that parenteral administration of the vitamin is necessary. In this study we report the biochemical and histological response in four patients with symptomatic PBC and osteomalacia to treatment with either parenteral vitamin D₂ or oral 25-OHD₃.

Methods

PATIENTS

The four patients, three female and one male, aged 50-66 years (mean 60 years) with symptomatic PBC and histological osteomalacia have been described elsewhere (Compston and Thompson, 1977). Patients 1 and 2 were treated with oral 25-OHD₃, 50 µg/day, and patients 3 and 4 with intramuscular vitamin D₂, 150 000 units once weekly (Table 1). All except patient 3 were taking cholestyramine (4 g twice or thrice daily).

Control values for bone histology were obtained from post-mortem biopsies of seven females and

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Table 1 Details of vitamin D therapy and quantitative bone histology before and after treatment

Patient	Treatment	Duration of treatment (months)	Osteoid volume % total cancellous volume		Osteoid surface % total cancellous surface		Calcification fronts % osteoid surface	
			Before	After	Before	After	Before	After
1	25-OHD ₃ *	5	21.3	2.7	64.3	40.1	8.4	79.2
2	25-OHD ₃ *	12	14.3	0	78.1	0	8.3	—
3	Vitamin D ₃ †	6	12.3	0.5	24.8	0.9	35.3	100
4	Vitamin D ₃ †	13	8.9	2.0	18.1	19.6	6.5	100
Normal mean ± SD			2.0 ± 1.7		17.9 ± 10.9		63.3 ± 4.5‡	

*By mouth.

†Intramuscularly.

‡Bordier *et al.* (1975).

19 males, aged 29-75 years (mean 53 years), who had died suddenly and had no history of immobilisation or metabolic bone disease.

BONE HISTOLOGY

Full-thickness iliac crest biopsies (Bordier *et al.*, 1964) were obtained before and after treatment. Undecalcified sections (8 µm) were stained by the Von Kossa technique and 1% toluidine blue. Calcification fronts were demonstrated by fluorescence microscopy of 10 µm unstained sections after oral administration of 900 mg demethylchlor-tetracycline 48 hours before the biopsy. Biopsies were quantified using a Zeiss 25 point eye-piece graticule.

BIOCHEMISTRY

Plasma 25-OHD concentrations were measured by a competitive protein-binding assay (Edelstein *et al.*, 1974) using normal human serum as binding protein. Serum calcium, phosphate, alkaline phosphatase, bilirubin, and albumin were measured on an SMA 12/60 autoanalyser, and serum calcium concentrations were corrected for the serum albumin level (Berry *et al.*, 1973). Urinary calcium was measured on an Eppendorf flame photometer.

Results

BONE HISTOLOGY (Table 1)

All patients responded well to vitamin D therapy, with a reduction in osteoid amount and increase in calcification fronts to normal values.

PLASMA 25-OHD (Table 2)

Plasma 25-OHD concentrations were below normal in all patients before vitamin D therapy. After five to 12 months' treatment all had levels within the normal range.

SERUM CALCIUM AND PHOSPHATE (Table 2)

Serum calcium and phosphate levels were higher after vitamin D therapy in patients 1, 3, and 4,

but, in patient 2, levels of calcium and phosphate fell during therapy, the phosphate to less than normal. After correction for her much reduced serum albumin, serum calcium after treatment was mildly raised in patient 1.

TWENTY-FOUR HOUR URINARY CALCIUM (Table 2)

Twenty-four hour urinary calcium excretion was below normal in all patients before treatment. The values after treatment, measured in patients 2, 3, and 4, were normal in patients 2 and 4, but remained low in patient 3.

SERUM ALKALINE PHOSPHATASE AND BILIRUBIN (Table 3)

After treatment with vitamin D, serum alkaline phosphatase had fallen in all patients, the greatest change occurring in patients 1 and 3. Serum bilirubin increased in all patients during the treatment period.

Discussion

This study has demonstrated that osteomalacia associated with symptomatic PBC may respond to oral or parenteral vitamin D, and that, even in the presence of severe liver disease, 25-hydroxylation of the parent vitamin, or intestinal absorption of orally administered 25-OHD₃, can be adequate to produce normal plasma 25-OHD levels and to promote bone healing, despite the continued cholestyramine therapy in three patients.

The value of histological examination of bone in the diagnosis of osteomalacia has recently been emphasised (Compston *et al.*, 1978), and our findings show that it is also useful in assessing the response to treatment, as biochemical measurements may be misleading. Thus, in patient 2, serum calcium and phosphate levels were lower after vitamin D therapy than before, and urinary calcium excretion remained low in patient 3 after bone healing had occurred.

Table 2 Serum calcium and phosphate, plasma 25-OHD, and 24-hour urinary calcium before and after vitamin D therapy

Patient	Corrected serum calcium (mmol/l)		Serum phosphate (mmol/l)		Plasma 25-OHD (nmol/l)		Urinary calcium (mmol/24 hr)	
	Before	After	Before	After	Before	After	Before	After
1	2.25	2.73	0.8	1.3	3.5	29.8	0.5	—
2	2.45	2.30	1.1	0.7	11.8	64.4	2.0	4.6
3	2.32	2.48	1.0	1.2	17.3	44.8	0	0.4
4	2.32	2.54	1.0	1.5	10.5	89.3	0.07	2.0
Normal range	2.25-2.55		0.8-1.4		25-100		2.5-7.5	

Table 3 Serum bilirubin, albumin, and alkaline phosphatase before and after vitamin D therapy

Patient	Serum bilirubin (μ mol/l)		Serum albumin (g/l)		Serum alkaline phosphatase (KAU/dl)	
	Before	After	Before	After	Before	After
1	28	460	36	27	140	56
2	24	30	40	44	72	54
3	216	400	41	41	91	33
4	58	164	36	31	79	65
Normal range	5-20		38-56		4-13	

However, the serum alkaline phosphatase concentration fell in all patients during treatment, especially in patients 1 and 3, despite deterioration of liver function, and although isoenzymes were not measured, this decrease in total alkaline phosphatase activity was probably due to a fall in the bone isoenzyme concentration. The normal plasma 25-OHD levels achieved by all patients during vitamin D therapy suggested that the dose administered was in the therapeutic range, although others have found that patients with normal or high-normal plasma 25-OHD concentrations may still have osteomalacia after many months' treatment (Long *et al.*, 1978a).

The prognosis of symptomatic PBC is poor (Sherlock, 1959), and in patients with advanced disease the diagnosis of osteomalacia may not be clinically important. None of our patients had severe bone pain or fractures, and two (1 and 4) have already died from their liver disease. However, three years after diagnosis patient 2 still has relatively mild, anicteric PBC, and treatment of her osteomalacia may well have prevented the onset of symptoms.

Recently there has been much interest in the therapeutic applications of vitamin D metabolites and their analogues. Parenteral 1,25-(OH)₂D₃ was claimed to heal osteomalacia in four patients with PBC (Long *et al.*, 1978b), although calcification fronts were not examined, and these patients had been previously treated with parenteral vitamin D. Five patients with PBC and osteomalacia were treated with oral 25-OHD₃ (Reed *et al.*, 1977) and all showed a reduction in osteoid surface, although calcification fronts were again not reported. Wagon-

feld *et al.* (1976) found that large oral doses (100-200 μ g/day) of 25-OHD₃ improved or stabilised bone mineral content in six of seven patients with PBC, whereas oral or subcutaneous vitamin D₂ was ineffective, but bone biopsy specimens were not examined, and the apparent lack of effect of vitamin D may have been due in some patients to the small doses given, and in others to the subcutaneous route of administration.

We conclude that both parenteral vitamin D₂ and oral 25-OHD₃ in the doses used in this study, may be effective in healing osteomalacia associated with primary biliary cirrhosis.

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