Parenteral 1,25-dihydroxycholecalciferol in hepatic osteomalacia

R G LONG, Z VARGHESE, E A MEINHARD, R K SKINNER, M R WILLS, S SHERLOCK

Summary and conclusions

Despite regular long-term parenteral vitamin D₃ treatment, four patients with biliary cirrhosis had multiple symptoms of bone disease and bone biopsy specimens showed osteomalacia without osteoporosis. Three patients also had a proximal myopathy. Plasma calcium values (after correction for albumin), phosphorus, magnesium, and serum 25-hydroxy-vitamin D were within normal limits. Treatment with 1,25-dihydroxycholecalciferol (1,25-(OH)₂D₃) relieved symptoms in three of the four patients and improved those in the fourth. Histological examination of bone showed improvement in all four patients, but serum and urinary biochemical changes were not pronounced.

We conclude that 1,25-(OH)₂D₃ treatment has a beneficial effect on bone and muscle in hepatic osteomalacia, either because vitamin D 1-hydroxylation fails in biliary cirrhosis or because hepatic osteomalacia is resistant to vitamin D₂ metabolites.

Introduction

Hepatic osteomalacia is a recognised complication of chronic cholestatic liver disease and may lead to severe bone pain and multiple fractures.¹⁻³ Until 1977 no consistently effective treatment for this condition had been described. Intravenous calcium gave temporary symptomatic relief of bone pain in patients with primary biliary cirrhosis (PBC), but the amount of osteoid tissue seen on bone histology was reduced in only one patient.¹ Parenteral vitamin D₃ treatment restores calcium-⁴⁴Ca) absorption and 25-hydroxy-vitamin D (25-OHD) concentrations to normal in PBC.⁴⁻⁶

In a series of 25 patients with cholestatic liver disease, 16 patients who had received regular parenteral vitamin D₂ treatment and usually had normal serum 25-OHD values had more osteoid tissue on quantitative bone histology than the nine patients who had not received vitamin D₃ supplements.¹ Vitamin D₃ (ergocalciferol) and D₃ (cholecalciferol) are 25-hydroxylated in the liver; further 1-hydroxylation to the most biologically active vitamin D metabolite occurs in the kidney.

It was thought that the normal 25-OHD values in the presence of osteomalacia might indicate a failure of 1-hydroxylation, even though the kidney rather than the liver is the primary site of 1-hydroxylation. We studied the effect of regular intramuscular doses of 1,25-dihydroxycholecalciferol (1,25-(OH)₂D₃) in four patients with biliary cirrhosis complicated by osteomalacia shown by biopsy.

Patients and methods

Some clinical details of the four women patients are summarised in table I. All four had histologically proved cirrhosis and evidence of portal hypertension complicated by gastro-oesophageal varices (in patient 1 these had first bled 10 years ago and in patient 2 a successful portacaval shunt had been performed 18 months before). All the patients had mild fluid retention, and three (cases 2-4) had mild to moderate portosystemic encephalopathy when untreated, and were pigmented. Plasma urea concentration and endogenous creatinine clearance values were normal. Daily calcium intake exceeded 450 mg in all four patients, and they had been regularly treated for at least three years with monthly or fortnightly intramuscular injections of 100,000 units of vitamin D₃ in ethyl oleate (Evans Medical); the latter was stopped when 1,25-(OH)₂D₃ treatment was started. All four patients had received cholestyramine for pruritus; patients 1 and 3 had not received any for five years and one year respectively. None of the patients had received long-term treatment with corticosteroids, frusemide, phenobarbitone, or other known inducing agents. No change in treatment or diet which was likely to affect bone state was made in the final year of vitamin D₃ treatment or after starting 1,25-(OH)₂D₃.

All four patients had generalised bone tenderness, and a radiological skeletal survey showed bone thinning but no evidence of osteitis fibrosa cystica or periosteal reactions. Patient 1 had had an intercostal fracture of the right femur treated by pin and plate six months earlier. Patient 3 had fractured the right medial malleolus three years before and right femoral head two years earlier; skeletal survey showed partial collapse of the third and fourth lumbar vertebrae and a fracture of the eighth rib posteriorly. The fourth patient had fractured the left neck of femur, left tibia and fibula, and right radius and ulna within the previous nine months after two falls; radiology showed healing fractures. She also had Sudeck's atrophy of the left foot and collapse of multiple lumbosacral vertebrae. Three patients (cases 2-4) had proximal muscle weakness, and in two (cases 2 and 3) myopathic changes were seen on electromyography.

1,25-(OH)₂D₃ was chemically synthesised, and ampoules of 15 μg in arachis oil were prepared under aseptic conditions. Every four weeks intramuscular injections of 15 μg 1,25-(OH)₂D₃ were given (except for the fourth patient, who once received 30 μg).

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Table I—Clinical details of women patients at start of treatment with 1,25-(OH)₂D₃

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>Duration of liver symptoms (years)</th>
<th>Duration of jaundice (years)</th>
<th>Serum total bilirubin (μmol/l)</th>
<th>Duration of vitamin D₃ treatment (years)</th>
<th>Duration of bone symptoms (years)</th>
<th>Proximal myopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>PBC</td>
<td>18</td>
<td>0</td>
<td>29</td>
<td>5</td>
<td>2</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>PBC</td>
<td>4</td>
<td>2</td>
<td>58</td>
<td>3</td>
<td>3</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>PBC</td>
<td>6</td>
<td>5</td>
<td>729</td>
<td>4</td>
<td>3</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>PBC</td>
<td>6</td>
<td>6</td>
<td>483</td>
<td>4</td>
<td>4</td>
<td>Present</td>
</tr>
</tbody>
</table>

PBC = Primary biliary cirrhosis; SBC = Secondary biliary cirrhosis.

Conversion: SI to traditional units—Serum bilirubin: 1 μmol/l = 0.058 mg 100 ml.
Full investigations were performed before starting 1,25-(OH)\textsubscript{2}D\textsubscript{3} and repeated in patients 1 and 2 after five months' treatment; plasma calcium and phosphorus were measured for two to three days after the injection, and again four weeks before the next injection. Biochemical results presented are from specimens taken one month after the previous injection. Venous blood samples were taken without stasis after a 12-hour overnight fast. Plasma concentrations of calcium, phosphorus, magnesium, alkaline phosphatase, total protein, albumin, creatinine, and ethanol-extractable hydroxyproline were estimated by standard methods.

Serum 25-OHD was measured by modified competitive protein binding\textsuperscript{11} and immunoreactive parathyroid hormone (PTH) by radioimmunoassay\textsuperscript{12}; interassay variation was avoided by doing all the assays in one batch. Two consecutive 24-hour urine collections on the fourth and fifth days of a standardised daily 800-mg calcium diet were tested for calcium, hydroxyproline, and creatinine excretion and the mean of the two results used. Phosphate absorption was assessed by an oral load of 17.4 g sodium phosphate crystals (BP) (1.5 g elemental phosphorus) dissolved in 200 ml water.\textsuperscript{13} A 10-Ci dose of \textsuperscript{47}Ca in 200 ml milk was given by mouth after overnight fasting, and calcium absorption measured by plasma content of isotope at three hours (as a percentage of the dose per litre plasma) and whole-body retention of dose at seven days.\textsuperscript{14} Both these absorption tests were performed one month after the previous injection of vitamin D metabolites.

Vertical biopsy specimens of bone from the iliac crest were taken under local anaesthesia with a Burkhart drill. Follow-up specimens were taken from all the patients, but those from patients 3 and 4 were taken after death. Specimens were processed by the method of Trippe and McKay.\textsuperscript{15} Longitudinal sections were stained by standard haematoxylin-and-eosin and Van Gieson techniques, and examined qualitatively and quantitatively by computer.\textsuperscript{16} The microscopical image of the trabecular portion of the section was projected (x100 magnification) and traced directly on to computer data cards. An optical mark reader fed the data into a minicomputer to provide tissue volume proportion (Vv\%) of osteoid, mineralised bone, and total bone, and surface extent (Sv\%) of osteoblasts and osteoclasts. No. usually osteoid tissue is less than 0.5% Vv and total bone is more than 20% Vv. Higher and lower values respectively indicate osteomalacia and osteoporosis.

Results
Some of the results of histological and biochemical investigations are summarised in table II.

**Clinical response**—Patients 3 and 4 died in liver failure eight and four weeks after the start of 1,25-(OH)\textsubscript{2}D\textsubscript{3} treatment respectively. Three patients (cases 1-3) lost all bone pain during the first two to three months of treatment, and in the fourth, bone pain had decreased at the time of death. Proximal myopathy had disappeared within three months in patients 2 and 3. No further fractures occurred in any of the patients. Patients 1 and 2 remained symptom-free after 14 and 12 months' 1,25-(OH)\textsubscript{2}D\textsubscript{3} treatment respectively.

**Bone histology**—All biopsy specimens of bone taken before 1,25-(OH)\textsubscript{2}D\textsubscript{3} treatment showed excess osteoid of up to 25% of bone matrix as "seams" 2-4 lacunae thick. Very few active osteoblasts or osteoclasts were seen. The trabeculae were within normal limits of size and spacing without woven bone. The pattern of layering\textsuperscript{1} was typical of normal or low rates of bone turnover. The mineralisation defect was therefore that of osteomalacia without osteoporosis. The amount of osteoid tissue decreased greatly in all four patients, showing that treatment of the bone mineralisation defect over one to five months had been successful. In patient 3 the second biopsy showed some areas of osteoclastic tunnelling resorption; this might have indicated subclinical overtreatment, and was not seen in the other patients. Otherwise osteoblasts and osteoclasts were as scant as in the first biopsy.

**Calcium, phosphorus, and magnesium metabolism**—Plasma calcium values after correction for albumin were initially normal despite the osteomalacia; all increased after 1,25-(OH)\textsubscript{2}D\textsubscript{3} treatment. The fourth patient was the first to be treated, and was given 1,25-(OH)\textsubscript{2}D\textsubscript{3} 30 \mu g intramuscularly; the plasma calcium rose to 3.15 mmol/l (12.6 mg/100 ml) on the third day, but fell to 2.83 mmol/l (11.3 mg/100 ml) the next day, after diuresis. No episodes of hypercalcaemia were seen, but plasma calcium tended to be higher by 0.13 mmol/l (0.5 mg/100 ml) to 0.25 mmol/l (1.0 mg/100 ml) the week before each injection of 15 \mu g 1,25-(OH)\textsubscript{2}D\textsubscript{3} and plasma phosphorus also rose by up to 0.31 mmol/l (1.0 mg/100 ml). Twenty-four-hour urinary calcium excretion tended to decrease with treatment.\textsuperscript{1} Ca absorption at three hours and retention at seven days were within normal ranges for three of the four patients before treatment; in patients 1 and 2 there was no detectable change with 1,25-(OH)\textsubscript{2}D\textsubscript{3} treatment. Plasma phosphorus values were in the low-normal range. Phosphorus absorption was normal in patients 2, 3, and 4 and mildly reduced in patient 1; treatment with 1,25-(OH)\textsubscript{2}D\textsubscript{3} did not change the absorption patterns in patients 1 and 2. Plasma magnesium values were initially all in the normal range, and tended to decrease with 1,25-(OH)\textsubscript{2}D\textsubscript{3} treatment.

**Vitamin D metabolism**—Serum 25-OHD values all decreased after stopping vitamin D\textsubscript{3} and starting 1,25-(OH)\textsubscript{2}D\textsubscript{3} treatment. After 11 and 9 months, serum 25-OHD levels had fallen to 16-9 pg/ml in patient 1 and 4-2 pg/ml in patient 2.

**Plasma alkaline phosphatase**—Values were raised in all four patients, and were in keeping with the predominant circulating isoenzyme that of hepatobiliary rather than bone origin. No definite trend occurred with treatment.

**Parathyroid function**—Plasma concentrations of immunoreactive PTH and hydroxyproline and 24-hour urinary hydroxyproline were within normal ranges for all four patients. Bone histology and radiology showed no signs of hyperparathyroidism.

**Discussion**
Our four patients had osteomalacia and normal parathyroid function, despite regular parenteral vitamin D\textsubscript{3} treatment and normal serum 25-OHD values. Nevertheless, they responded to

| Table II: Summary of results of histological and biochemical investigations in four patients before and after treatment with 1,25-(OH)\textsubscript{2}D\textsubscript{3} |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Osteoid tissue (Vv%)** | **Plasma calcium (mmol/l)** | **Plasma corrected* calcium (mmol/l)** | **24-h urinary calcium (mmol/l)** | **% retention of 3\textsuperscript{25}Ca at 7 days** | **Plasma phosphorus (mmol/l)** | **Phosphorus absorption** | **Plasma magnesium (mmol/l)** | **Serum 25-OHD (pg/ml)** | **Plasma alkaline phosphatase (KAU/100ml)** |
| Normal range: | <0.5 | 2.13-2.63 | 2.13-2.63 | 2.5-7.5 | 10-35 | 0.81-1.45 | 0.70-1.00 | 9.44 | 3-13 |
| **Case 1:** | | | | | | | | | |
| On vitamin D\textsubscript{3} | After 4 months | of 1,25-(OH)\textsubscript{2}D\textsubscript{3} | | | | | | | |
| | | 2.05 | 2.20 | 5.3 | 20.3 | 1.10 | Reduced | 0.80 | 24.3 | 24 |
| | | 2.10 | 2.28 | 3.4 | 28.2 | 1.26 | Reduced | 0.88 | 21.1 | 20 |
| | | 2.35 | 2.40 | 6.5 | 33.1 | 0.84 | Normal | 0.75 | 11.0 | 57 |
| | | 2.30 | 2.45 | 2.0 | 23.4 | 0.94 | Normal | 0.66 | 7.1 | 39 |
| **Case 2:** | | | | | | | | | |
| On vitamin D\textsubscript{3} | After 5 months | of 1,25-(OH)\textsubscript{2}D\textsubscript{3} | | | | | | | |
| | | 2.38 | 2.35 | 1.3 | 9.0 | 1.10 | Normal | 0.88 | 17.3 | 101 |
| | | 2.35 | 2.40 | 1.6 | 16.2 | 1.00 | Normal | 0.81 | 11.4 | 55 |
| | | 2.43 | 2.80 | 1.6 | 11.3 | 0.81 | 10.1 | 80 |

*Corrected for variations in albumin concentration.

Conversion: SI to traditional units—Plasma calcium: 1 mmol/l = 4 mg/100 ml. Urinary calcium: 1 mmol/l = 40 mg. Plasma phosphorus: 1 mmol/l = 3-10 mg/100 ml. Plasma magnesium: 1 mmol/l = 24-140 mg/100 ml.
1,25-(OH)₂D₃ treatment, bone and muscle symptoms being particularly improved and the volume proportion of osteoid tissue visible on bone biopsy reduced. Plasma biochemical values and intestinal calcium and phosphorus absorption were either normal or only slightly disturbed at the start of the study; treatment resulted in only mild improvement of the plasma calcium values. Phosphate depletion might have contributed to the osteomalacia seen in these patients, but subnormal plasma phosphorus values would be expected if it was a major factor. The lack of improvement in calcium and phosphorus absorption after 1,25-(OH)₂D₃ may have been due to the timing of the test: one month after the previous injection, the 1,25-(OH)₂D₃ may have been largely excreted. The post-treatment fall in urinary calcium excretion might have been caused by greater bone utilisation of calcium. In view of the initial normality and the minimal change in plasma biochemical values after treatment, the effects on bone and muscle might be attributed to a direct effect of 1,25-(OH)₂D₃; alternatively, the rise in circulating plasma calcium might have improved bone calcification.

In chronic renal failure the physiological long-term replacement dose of 1,25-(OH)₂D₃ is about 0-5-1.0 μg/day by mouth; this has resulted in resolution of osteomalacia and secondary hyperparathyroidism. In our experience, osteomalacia occurs despite normal serum 25-OHD values; the fall in 25-OHD values in our patients and improved histological appearances of bone suggest that 25-OHD is not of first importance in bone mineralisation. 1-Hydroxylation may fail in biliary cirrhosis as in renal disease; this, however, seems to have been relatively unlikely in our patients as renal excretory function was normal. Alternatively, vitamin D₂ metabolites may be less effective in healing hepatic osteomalacia than vitamin D₃ metabolites. This has been previously described in animals. In one series oral 25-OHD stabilised or improved bone mineral content in six or seven patients with PBC, but the underlying bone disease was unknown as histological examination of bone was not performed; the authors did not state whether the 25-OHD originated from vitamin D₂ or D₃. A subsequent abstract from the same group has shown reduction in osteoid tissue in six patients with PBC treated with the same metabolite.

Our study shows that the histological osteomalacia of chronic cholesstatic liver disease is dependent on defective metabolism of vitamin D. Further research is required to elucidate the nature of defect(s) and the best metabolite and dose to prevent and correct hepatic osteomalacia. If the response to treatment in these four patients was due to a differential response to vitamin D₂ and D₃ metabolites, hepatic osteomalacia might be prevented and healed with vitamin D₃; if, alternatively, there was a failure of 1-hydroxylation, then 1,25-(OH)₂D₃ would be the treatment of choice. The mechanism of the ‘healing’ response to 1,25-(OH)₂D₃ in this study was not apparent in view of the minimal change in measured biochemical variables—various factors may have been playing a part. Nevertheless, vitamin D metabolites may have a direct beneficial effect on bone and muscle in hepatic osteomalacia.

We thank Dr N T Pollitt of Roche Products Ltd, Welwyn Garden City, for providing the 1,25-(OH)₂D₃; Miss L Goodwill for preparing the 1,25-(OH)₂D₃ for parenteral use; Mr S Newman for performing the ⁴⁰Ca absorption tests; Dr J M Zanelli for advice; and the National Institute of Biological Standards and Control for providing reagents for radioimmunassy of immunoreactive PTH. We thank Mr A. Chester Beatty for the financial support of RL and the Medical Research Council for the financial support of EM.

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References

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SIDE EFFECTS OF DRUGS

Tardive dyskinesias associated with metoclopramide

Metoclopramide is widely used for relief of upper gastrointestinal symptoms such as heartburn, nausea, and vomiting. Adverse reactions are relatively rare,1 but may include disturbances of the central nervous system such as drowsiness, restlessness, and extrapyramidal reactions.2 Acute facial dyskinesias3 and dystonia3,4 have been reported in several children and young adults shortly after administration of low doses of metoclopramide. These extrapyramidal effects were attributed to idiosyncrasy, and completely subsided in all cases after withdrawal of the drug. We report on a patient who developed tardive dyskinesia after long-term treatment with high doses of metoclopramide. We know of no other report of such an association.

Case report

A 48-year-old Jewish man began to complain of persistent nausea without vomiting or other gastrointestinal symptoms. No organic basis was found for his complaint, and results of physical and neurological examinations as well as various laboratory tests were normal. There was no evidence of other disease or metabolic abnormalities. There was no history of food or drug allergies in the patient or in his family. Metoclopramide (Pramin, Israeli Laboratories, Israel) was prescribed for the nausea. He took the drug by mouth at a daily dose of 20-40 mg for about six years. He asked for the dosage to be gradually increased, and during the last four years he con-