Dialysis Bone Disease in Childhood: Treatment with 25-Hydroxycholecalciferol

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Summary

The effect of 8 months of administration of moderate doses of 25-hydroxycholecalciferol (25 OH D3) on radiologic, biologic, and bone histologic changes was assessed in five children on chronic hemodialysis. Osteomalacia, defined by an increase in the thickness index of the osteoid seams and decrease of the calcification rate, was present on the initial bone biopsy of only one patient and improved with the treatment. Secondary hyperparathyroidism and its prints on bone tissue, noted in all five children, did not improve in the absence of adequate serum phosphorus control. Furthermore, cancellous bone volume diminished in two patients with the administration of 25 OH D3. This activity of the drug could be related to its inhibitory effect of osteoblastic apposition as demonstrated by the decrease in the calcification rates, while the thickness index of the osteoid seams remains normal.

Despite the small number of patients studied, these results suggest the importance of limiting the prescription of 25 OH D3 to children suffering from renal osteodystrophy only after having assessed unequivocally an osteomalacic component by histodynamical criteria. Secondary hyperparathyroidism appears not to be improved with moderate doses of 25 OH D3 in the absence of adequate serum phosphorus control.

Speculation

Treatment with 25 OH D3 may be justified for the treatment of chronic hemodialyzed children with definite osteomalacia as a component of their renal osteodystrophy. Secondary hyperparathyroidism appears not to be improved with moderate doses of 25 OH D3 in the absence of adequate serum phosphorus control.

Only a few studies have been performed on the treatment of renal osteodystrophy with vitamin D metabolites in chronic hemodialyzed children (17, 21) and none has included a dynamic histologic analysis based on tetracycline double labeling. The effect of 8 months administration of moderate doses of 25 OH D3 on radiologic, biologic, and bone histologic changes was assessed in five children on chronic hemodialysis.

PATIENTS AND PROCEDURE

Five children, 11- to 19-yr-old, were included in this study. All presented with renal osteodystrophy as estimated by clinical and radiologic data, serum immunoreactive parathyroid hormone (iPTH) levels and histologic criteria (Tables 1-3). Details of these findings have been given elsewhere (8, 12). They were dialyzed for 5 hr three times a week using a calcium dialysate of 1.75 mmole/liter. Before treatment with 25 OH D3, each child was given daily 25 mmole (1 g) of calcium, 125 nmole (2000 IU) of vitamin D2, and 7.5-11.5 mmole (600-900 mg) of aluminium hydroxide.

The synthetic 25 OH D3 (Laboratoires Roussel, Paris, France) was given orally, once a day during 8 months. Seventy-five nmole (30 µg) of 25 OH D3 were administered, daily during the first 3

months of the study; then, the dose was increased to 125 nmole (50 µg) daily during the next 5 months. During the study, calcium supplements and vitamin D2 were discontinued. The aluminium hydroxide was followed at the same dose. No patient was receiving anticonvulsant drugs.

METHODS

PLASMA BIOCHEMISTRY

All blood samples were obtained immediately before dialysis. Total plasma calcium was measured by atomic spectrophotometry (Perkin Elmer), plasma phosphorus, and alkaline phosphatases (1) with autoanalyzer (Technicon, Daumont, France). Serum iPTH levels were measured by radioimmunoassay using a guinea-pig antibody (GP6) and identifying the carboxy terminal of PTH (2, 5). Serum 25-hydroxy vitamin D (25 OHD) concentrations were measured using a radiocompetitive protein binding assay (18).

RADIOLOGIC EVALUATION

The subperiosteal resorption was looked for on X-ray examinations of the phalanges and the skull. Osteomalacia was assessed on radiograms of the wrist and the tibiae and osteosclerosis on the third lumbar vertebra. Bone maturity was measured according to Greulich and Pyle (11).

BONE HISTOMORPHOMETRY

Bone tissue was obtained before and contralaterally 8 months after 25 OH D3 therapy. Transfixing iliac bone biopsies were performed on patients with an 8 mm internal diameter trephine, after tetracycline double labeling, consisting of a dose of 15 mg/kg/day of Declomycin given for 2 days and, after an interval of 12 days, repeated for 4 days. The biopsy site was situated about 2 cm below the top of the iliac crest and 2 cm behind the anterosuperior iliac spine.

The undecalcified bone samples were embedded in Bioplastic Ward's and 10 longitudinal nonconsecutive serial sections, 8 µm thick, were cut with a microtome Jung K. Four sections were stained with Solochrome cyanin R, four with Goldner's stain and two with Toluidine blue. Four additional unstained sections, 20 µm thick, were used for the analysis of tetracycline labels, under fluorescent light.

The following parameters were measured: 1) Trabecular bone volume (T.B.V.) that represents the fraction of the cancellous bone volume occupied by bone matrix (%). 2) Trabecular osteoclastic resorption surface (T.O.R.S.), that represents the percent of the total trabecular bone surface occupied by resorption sites (that notch the trabecular edges) (%). We did not distinguish between "active" and "inactive" resorption surfaces, according to the presence or absence of osteoclasts in the Howship's lacunae. 3) Number of osteoclasts per mm² of bone tissue (section) (Ocl./mm²). 4) Trabecular osteoid volume (T.O.V.) that represents the percent of a given volume of the trabecular bone occupied by osteoid (%). 5)

Table 1. *Clinical data*

Patients				Duration		Height (cm)		Bone age (yr)	
N	Sex	Age (yr)	Diagnosis	Renal failure (yr)	Hemodialysis (months)	treatment		treatment	
						Pre	Post	Pre	Post
1	F	13	Renal hypoplasia	11	44	119.5	122.5	7 ¹⁰ / ₁₂	8 ¹⁰ / ₁₂
2	F	11	Membrano-proliferative glomerulonephritis	4	40	120	123	7 ¹⁰ / ₁₂	8 ¹⁰ / ₁₂
3	F	19	Oligonephronic hypoplasia	17	32	142.5	142.5	Adult	
4	M	18	Uropathy	3	28	165	165	Adult	
5	M	13	Membranous glomerulonephritis	4	16	140.5	141	12 ⁹ / ₁₂	12 ⁹ / ₁₂

Table 2. *Biologic data before and after 25 OH D3 therapy*

Plasma					
Patients N	Ca (mmole/liter)	P (mmole/liter)	Alkaline phosphatase (IU/ml)	PTH (μEq/ml)	25 OHD (nmole/liter)
1 Control	2.37	1.84	68	222	26
After 25 OH D3	2.40	2.54	36	465	135
2 Control	2.12	3.06	72	325	39.50
After 25 OH D3	2.55	2.29	25	340	250
3 Control	2.27	2.19	42	440	52.50
After 25 OH D3	2.32	1.51	22	490	250
4 Control	2.65	2.70	22	185	26.50
After 25 OH D3	2.67	3.25	35	270	250
5 Control	2.62	2.64	25	76	54
After 25 OH D3	2.40	3.03	25	405	140
Normal range	2.07–2.62 (n = 13)	0.96–1.77 (n = 13)	9–35 (n = 13)	0–50 (n = 35)	12.0–61.5 (n = 39)
Age range of the control subjects (yr)		10–15	10–15	Adults	20–35

Trabecular osteoid surfaces (T.O.S.) that represents the percent of the total trabecular surfaces covered by osteoid (%). 6) Thickness index of the osteoid seams (T.I.O.). This index is calculated by the ratio T.O.V./T.O.S. $\times$ 100. 7) Calcification rate (C.R.) was evaluated in cancellous bone by measuring the mean distance between the two fluorescent labels on each site of active calcification (10).

RESULTS

CLINICAL AND RADIOLOGIC RESULTS

The five children had no deformities, bone pain, or epiphyseolysis. Growth retardation was present in three cases (patients 1–3) (Table 1) and 25 OH D3 had little effect on growth velocity that never exceeded 3 cm/yr. There were no extrasosseous calcifications, except conjunctival calcifications that were revealed on slit lamp examination at the beginning of treatment in three children (patients 3–5) and were present at the end of the study in four cases (patients 1, 2, 4, 5). Subperiosteal resorption was noted in four cases (patients 1–4) and osteosclerosis in two (patients 3, 4). No change of these radiologic signs was noted as an affect of the treatment. Classical radiologic signs of osteomalacia or rickets were never observed. Bone age increased by 1 yr in two of the children during the study (patients 1, 2).

BIOLOGIC RESULTS (TABLE 2 AND FIGURE 1)

Plasma calcium, phosphorus, and alkaline phosphatase were checked regularly every month and the results are given in Figure 1. Plasma calcium remained within the normal range during the treatment, except in one child who had transient hypercalcemia (patient 2). In this case, treatment was temporarily discontinued. Plasma phosphate levels were elevated in all cases despite aluminum hydroxide, and there was no consistent change in serum phosphorus concentrations after 25 OH D3, except in patient 3 whose plasma levels returned to within the normal range after 6 months of treatment. Plasma alkaline phosphatase concentrations were initially elevated in three children (patients 1, 2, and 3) and returned gradually to within normal range. Plasma iPTH increased in the five children, but the variation of the mean level was not significant ($P < 0.1$). Circulating 25 OHD levels were all normal before the study and rose significantly ($P < 0.001$) in every case.

HISTOMORPHOMETRIC RESULTS (TABLE 3)

At the beginning of the study, all five children had histomorphometric abnormalities. The classical signs of hyperparathyroidism (increase of the trabecular osteoclastic resorption surfaces and of the number of osteoclasts per mm²) were seen to varying degrees in all five cases. With the treatment, hyperparathyroid signs did

Table 3. *Histometric data before and after 25 OH D3 therapy*

Patients <i>N</i>	T.B.V. (%) (± 4) ¹	T.O.R.S. (%) (± 2) ¹	Ocl/mm ² (± 0.2) ¹	T.O.V. (%) (± 1.5) ¹	T.O.S. (%) (± 6) ¹	T.I.O. (± 4) ¹	Calcification rate ($\mu\text{m/d}$) in cancellous bone (± 0.12) ¹
1 Control	15.9	21.4	2.60	19.8	68.8	28.8	0.98
After 25 OH D3	19.1	17.9	2.08	14.9	66.4	22.4	0.99
2 Control	18.6	25.4	3.40	9.3	50.6	18.4	1.64
After 25 OH D3	21.4	25.7	4.07	10.6	56.2	18.9	1.23
3 Control	36.1	18.7	2.80	9.4	46.8	20.0	1.56
After 25 OH D3	23.7	21.8	2.34	7.9	52.8	15.0	0.76
4 Control	45.8	10.3	1.2	5.4	39.9	13.6	1.26
After 25 OH D3	15	8.6	0.79	7.9	48.9	16.2	1.16
5 Control	12.7	9.2	0.40	4.6	29.4	15.0	0.74
After 25 OH D3	11.3	5.7	0.30	10.1	65.5	15.5	0.49
Normal controls (mean $\pm$ SD)							
Male	23.0 $\pm$ 4.1 (<i>n</i> = 11)	4.0 $\pm$ 1.3 (<i>n</i> = 5)		3.9 $\pm$ 2.3 (<i>n</i> = 19)	18.2 $\pm$ 9.3 (<i>n</i> = 19)	20.6 $\pm$ 4.2 (<i>n</i> = 19)	0.73 $\pm$ 0.13 (<i>n</i> = 42)
Female	22.9 $\pm$ 4.5 (<i>n</i> = 5)	3.8 $\pm$ 0.9 (<i>n</i> = 3)		2.1 $\pm$ 1.4 (<i>n</i> = 10)	11.0 $\pm$ 6.8 (<i>n</i> = 10)	19.3 $\pm$ 3.1 (<i>n</i> = 10)	
Age range of the control sub- jects (yr)	15–19			10–29		Adults	

¹ This SD is an approximation taking into account the intraobserver error and the sampling variation.

not change significantly. Osteomalacia, defined by an increase in the thickness index of the osteoid seams and decrease of the calcification rate (13), was present on the initial bone biopsy of only one patient (1) and improved with the treatment.

In the four other cases, the increase of the trabecular osteoid surfaces without modification of the calcification rate and of the thickness index, did not indicate osteomalacia, but only hyperparathyroidism, according to previous studies (13). Under the treatment, the trabecular osteoid surfaces increased in all these cases, while the calcification rate decreased. As the thickness index remained unchanged, the decrease in calcification rate was only due to a decrease in osteoblastic appositional rate, in other words, to a decrease in the rate of synthesis of new collagen by osteoblasts.

The trabecular bone volume was initially increased in two children (patients 3 and 4) and decreased in a third child (patient 5). Under the treatment, the trabecular bone volume diminished in the two patients with osteosclerosis. The surface of bone section occupied by woven bone decreased markedly under treatment. This decrease was assessed on qualitative criteria because the quantification of woven bone is imprecise.

DISCUSSION

Few studies have been made on the treatment of dialysis bone disease with 25 OH D3 (9, 19, 21) and only one concerned children (21). Antirachitic effect with pharmacologic doses of 25 OH D3 has been previously demonstrated in renal osteodystrophy (3, 7, 9, 19, 21). Moderate doses of this drug administered over a short period of time improved calcification front (7). It has been suggested that 25 OH D3 in physiologic concentrations can have an effect on bone mineralization in chronic renal failure (6). Eight anephric patients have shown no histologic evidence of osteomalacia up to 6 yr after bilateral nephrectomy (3). Eastwood *et al.* (6) noted a defect of mineralization only in patients with chronic

renal failure in whom plasma 25 OHD levels were low. However, this result was not confirmed in another study (15). It seems possible that 25 OH D3 is directly involved in active mineralization. The selective incorporation of tritiated 25 OH D3 in epiphyseal cartilage and bone growing rats has been reported (20).

Hypercalcemia may be seen during 25 OH D3 treatment. This disorder required, in a previous study, an interruption of the treatment and several injections of calcitonin; in one case, subtotal parathyroidectomy was performed (21).

The improvement of hyperparathyroidism is less evident. A drop in serum iPTH levels was absent in this study and insignificant in others (7, 9). Hyperphosphoremia prevents perhaps this decrease (9). A rise in phosphoremia has been previously reported during treatment with 25 OH D3 (9) or 1- α -hydroxycholecalciferol (9, 16). It may be explained by an increase in the intestinal absorption of phosphate, demonstrated during treatment with 1- α -hydroxycholecalciferol (16). In this study, secondary hyperparathyroidism and its prints on bone tissue, noted in all five children, did not improve in the absence of adequate serum phosphorus control. However, controversial results have been reported with a decrease in circulating iPTH (19) and in hyperparathyroidism bone lesions (19, 21). In these studies, plasma phosphorus levels were variable (21) or not reported (19). In addition, it is possible that, in these cases, the doses of 25 OH D3 used might not have been large enough because serum 25 OHD levels failed to rise above 135–250 nmole/liter during the treatment.

Furthermore, cancellous bone volume diminished in two patients with the administration of 25 OH D3. This was noted in a previous study (21). This activity of the drug could be related to its inhibitory effect of osteoblastic apposition. The inhibition of osteoblastic apposition is demonstrated in our cases by the decrease in the calcification rates, while the thickness index of the osteoid seams remains normal. This fact coincides with a marked decrease of the areas occupied by woven bone. Woven bone is

Fig. 1. Effects of 25 OH cholecalciferol on plasma calcium, phosphorus, and alkaline phosphatase; shaded areas represent normal ranges.

rapidly synthesised by osteoblasts and the osteoblastic appositional rate in woven zones is about twice normal as shown in Paget's disease of bone (14), fracture callus, or osteosclerotic metastases.

CONCLUSION

Despite the small number of patients studied, these results suggest the importance of limiting the prescription of 25 OH D3 to children suffering from renal osteodystrophy only after having assessed unequivocally an osteomalacic component by histodynamical criteria.

Secondary hyperparathyroidism appears not to be improved with moderate doses of 25 OH D3 in the absence of adequate serum phosphorus control.

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