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CLINICAL INVESTIGATION

$1,25(OH)_2D_3$ administration in moderate renal failure: A prospective double-blind trial

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$1,25(OH)_2D_3$ administration in moderate renal failure: A prospective double blind trial. This study represents the first randomized prospective, double-blind, placebo-controlled trial of the efficacy of $1,25(OH)_2D_3$ on bone histology and serum biochemistry in patients with mild to moderate renal failure. Sixteen patients with chronic renal impairment (creatinine clearance 20 to 59 ml per min) received either $1,25(OH)_2D_3$, at a dose of 0.25 to 0.5 μg daily (eight patients), or placebo. Transiliac crest bone biopsies were performed before entrance into the study and after 12 months of experimental observation. None of the patients were symptomatic or had radiological evidence of bone disease. Of the thirteen patients who completed the study, initial serum $1,25(OH)_2D$ levels were low in seven patients and parathyroid hormone levels were elevated in seven patients. Bone histology was abnormal in all patients. $1,25(OH)_2D_3$ treatment was associated with a significant fall in serum phosphorus and alkaline phosphatase concentrations as well as with histological evidence of an amelioration of hyperparathyroid changes. In contrast to previous reports, no deterioration of renal function attributable to the treatment occurred, perhaps because a modest dose of $1,25(OH)_2D_3$ was employed combined with meticulous monitoring. Further investigation is required to determine whether alternative therapeutic strategies (smaller doses or intermittent therapy) may avoid the potential for suppressing bone turnover to abnormally low levels in the long term.

Bone disease and the need for parathyroid surgery continue to bedevil patients with chronic renal failure. Prevention of bone disease and hyperparathyroidism with vitamin D metabolites appears logical, but long-term placebo-controlled studies in patients with mild to moderate renal impairment are not available. The few published prospective placebo-controlled studies have involved patients with end-stage renal failure [1, 2]. Histological evidence of bone disease is present early in the evolution of chronic renal failure [3] as is evidence of reduced vitamin D effects on the target organs, bone and gut [4, 5]. Blood levels of $1,25(OH)_2D$ have been reported previously to be normal or high [6–8]. More recent reports indicate serum $1,25(OH)_2D$ levels to be low in mild to moderate renal impairment [9, 10] and to be inversely correlated with glomerular filtration rate [11]. These values must, however, be interpreted in the light of the biochemical derangements associated with mild to moderate renal impairment, under which circumstance elevated serum levels of $1,25(OH)_2D$ would be appropriate [12].

It appears logical, therefore, to substitute $1,25(OH)_2D_3$ in patients with mild to moderate renal impairment, but evidence is scant as to whether treatment with this agent at this early stage is of benefit. Treatment involves the risks of hypercalcemia, and it has been claimed [13] that an adverse effect upon renal function may occur even in the absence of hypercalcemia. Consequently, we have carried out a one year, double-blind placebo-controlled trial of $1,25(OH)_2D_3$ in patients with mild to moderate renal impairment in an attempt to define whether a beneficial effect upon serum biochemistries and histological abnormalities can be obtained and, in particular, whether this can be achieved without any deleterious effects upon renal function.

Methods

Protocol

Sixteen subjects were recruited from the nephrology outpatient clinic at St. Bartholomew's Hospital. Patients with a creatinine clearance of 20 to 60 ml/min were eligible for the study. Exclusion criteria included: pregnancy, hypercalcemia, renal stones, poorly controlled hypertension, gastrointestinal or liver disease, urinary protein output greater than 3 g daily, psychosis, known tetracycline allergy, treatment with medication known to affect bone (anticonvulsants, heparin, corticosteroids) or vitamin D metabolites in pharmacological doses within the previous six months. Seventy-seven patients were screened, of whom 30 met the inclusion criteria. Sixteen agreed to take part in the study, and written informed consent was obtained. The study was approved by the St. Bartholomew's Hospital and Medical College Ethics Committee.

Three patients were withdrawn from the study: One, on active treatment, had a hypersensitivity reaction to tetracycline and was withdrawn within one week of starting the study; a second (on placebo), suffered a myocardial infarction at 40 weeks; in the third patient (on placebo) no satisfactory bone biopsy was obtained at 12 months. The results of these patients are not included. Individual patient data are shown in Table 1.

Patients continued with their usual antihypertensive medication throughout the study. One patient (Pat. #15) received thyroxine replacement and was clinically and biochemically

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Table 1. Age, sex and diagnosis of patients with moderate renal failure receiving $1,25(OH)_2D_3$ or placebo for 12 months

Pt. no.	Age	Sex	Diagnosis	Therapy
1	58	F	Uninephrectomy, glomerulonephritis	$1,25(OH)_2D_3$
3	64	F	Membranoproliferative glomerulonephritis	$1,25(OH)_2D_3$
5	52	M	Glomerulonephritis	$1,25(OH)_2D_3$
6	63	M	Membranous glomerulonephritis	Placebo
8	31	M	Uretero-pelvic junction obstruction	Placebo
10	40	M	Tubulointerstitial disease	Placebo
11	56	M	Glomerulonephritis	$1,25(OH)_2D_3$
12	36	M	Glomerulonephritis	Placebo
13	61	F	Cortical necrosis	Placebo
14	55	F	Hypertensive nephrosclerosis	Placebo
15	63	F	Hypertensive nephropathy	$1,25(OH)_2D_3$
16	52	M	Glomerulonephritis	$1,25(OH)_2D_3$
17	51	F	Glomerulonephritis	$1,25(OH)_2D_3$

euthyroid throughout the study. Oral aluminum containing phosphate binders were required only in one patient (Pat. #11). A renal dietician assessed all the patients before entry to the study in order to ensure an oral calcium intake of 800 mg daily—oral calcium supplements being taken when necessary in the morning (Pats. #1, 3, 6, and 15). No specific instructions as to protein and phosphorus intake were given but patients were instructed to continue their usual diet throughout the study. There is no evidence that any patient restricted protein intake to a great extent after commencing the study.

The study was designed as a double-blind placebo-controlled study lasting 12 months. Patients were allocated randomly to receive $1,25(OH)_2D_3$ or a matching placebo. The initial dose of $1,25(OH)_2D_3$ was 0.25 μg daily. If serum calcium remained below 2.6 mmol/liter and the urinary calcium was less than 7 mmol/24 hr, the dose was doubled between four and eight weeks after commencement of treatment. If hypercalcemia and/or hypercalciuria occurred the trial medication was stopped. When serum and/or urine calcium had returned to normal, treatment was recommended at half the previous dose. To rule out substrate deficiencies, all patients received 400 IU of vitamin D_3. Initial assessment included: history and physical examinations, urinalysis, and measurement of fasting total serum calcium (Ca), phosphorus (P), creatinine (Cr), alkaline phosphatase (AP), parathyroid hormone (PTH), 25 hydroxycholecalciferol, 1,25 dihydroxycholecalciferol, and creatinine clearance (mean of two measurements). Twenty-four hour urinary calcium and phosphorus excretions were measured on two occasions before commencement of the study. X-rays of hands, feet, clavicles and skull were carried out as was an initial bone biopsy.

Patients were seen at a minimum of four weekly intervals in a special research clinic. At each attendance symptoms were noted, blood pressure checked, and fasting blood drawn for all serum and urine biochemical parameters measured at baseline. Radiographs were repeated at 6 and 12 months, and the iliac crest bone biopsy repeated at 12 months.

Biochemical measurements

Serum and urine calcium, phosphorus and creatinine concentrations, and serum alkaline phosphatase concentration were measured by standard autoanalyzer methods.

Parathyroid hormone was estimated using a C terminal radioimmunoassay from Immuno Nuclear Corporation (Stillwater, Minnesota, USA) [14]. The coefficient of variation of this method was $< 10\%$ and normal values < 0.9 μg/ml.

Blood levels of $1,25(OH)_2D$ were measured with a modification of the method described previously [15]. The technique involved lipid extraction from 1 to 2 ml serum samples using acetonitrile and disposable C-18 columns (Waters Associates, Milford, Massachusetts, USA) followed by automated high-pressure liquid chromatography (HPLC) with Silica column (Waters Associates). The HPLC fraction containing $1,25(OH)_2D$ was measured in duplicate by competitive binding assay utilizing a semipurified receptor from calf thymus [16]. Intraassay variation was 5%; interassay variation was 9%.

Blood levels of 25(OH)D were measured with a competitive binding assay that utilizes rat serum [17]. Intraassay variation was 5%; interassay variation was 10%.

Bone histology and histomorphometry

Patients received tetracycline hydrochloride (Acromycin) 250 mg four times daily for two days. Ten days thereafter, they received demeclotetracycline (Declomycin®), 150 mg, four times daily for four days. After a further interval of four days, a transiliac crest bone biopsy was performed.

Bone samples were fixed in ethanol, dehydrated, and embedded in methylmethacrylate for mineralized bone histology. Serial undecalcified sections of 3- and 7-μm thickness were cut using a Reichert Jung microtome (Model 1140, Reichert Scientific Co., Buffalo, New York, USA). Three-μm thick sections were stained with the modified Masson-Goldner trichrome stain, which permits discrimination between mineralized and nonmineralized bone and gives excellent cellular detail [18].

Seven-μm thick unstained sections were prepared for phase contrast and fluorescent light microscopy. In addition, 7-μm thick sections were stained with the aurin tricarboxylic acid stain for detection of aluminum [19]. Static and dynamic parameters of bone structure, bone formation, and resorption were measured using the Osteoplan system (Carl Zeiss, Thornwood, New York, USA) as previously described [20].

The following histomorphometric parameters were obtained: (a) cancellous bone mass, that is, percentage of total bone occupied by trabecular bone; (b) mean trabecular diameter; (c) mean trabecular plate density, that is, cancellous bone mass $\times$ 10 per mean trabecular plate thickness; (d) mean wall thickness, the mean distance between cement lines and the trabecular surfaces of completed structural units; (e) lamellar osteoid volume, the percentage of bone occupied by lamellar osteoid; (f) woven osteoid volume, the percentage of bone occupied by woven osteoid; (g) lamellar osteoid surface, the percentage of trabecular surface covered by lamellar osteoid; (h) woven osteoid surface, the percentage of trabecular surface covered by woven osteoid; (i) mean thickness of lamellar osteoid; (j) bone-osteoblast interface, that is, percentage of trabecular surface covered by osteoblasts; (k) osteoblastic index, the number of osteoblasts per 100 mm trabecular boundary length

(only plump cuboidal cells juxtaposed to bone and arranged in a palisade-like manner were included in the measurement); (l) bone-osteoclast interface, the percentage of trabecular surface covered by mono or multinucleated osteoclasts; (m) osteoclastic index, the number of osteoclasts per 100 mm trabecular boundary length; (n) mineral apposition rate, the mean distance between tetracycline labels × 0.73/day of labeling-free interval; (o) double-labeled trabecular surface, the percentage of trabecular surface exhibiting two distinctively separated labels; (p) singly-labelled trabecular surface, the percentage of trabecular surface exhibiting single labels; (q) mineralization lag time, the mean osteoid seam thickness divided by mineralization rate; (r) osteon remodeling time (days) per osteoclast in unit time; and (s) stainable bone aluminum, that is, fraction of trabecular surface exhibiting aluminum deposits at the bone-osteoid interface. At least fifty optical fields were evaluated at a magnification of ×200 using an objective with 0.4 numerical aperture. All slides were read without knowledge of biochemical results or mode of therapy.

Statistics

Data on serum calcium, phosphorus, alkaline phosphatase, creatinine, parathyroid hormone, $25(OH)D_3$, $1,25(OH)_2D$, creatinine clearance, urinary calcium, phosphorus and urinary creatinine were analyzed by analysis of covariance, the initial value being used as the covariate.

Analysis was performed using multiple regression analysis, using the "Minitab" statistical package. Serum creatinine concentration was not normally distributed, so logarithmic values of creatinine were used for analysis of covariance.

Comparison between initial and final values of histomorphometric parameters of bone was made using the Wilcoxon Signed Rank Test for non-parametric data. Comparison between experimental and normal histomorphometric data was done using the Kruskall-Wallis analysis of variance.

Results

Compliance with treatment was excellent throughout the study as judged by repeated direct questioning of patients. No patient reported any adverse symptoms attributable to treatment during the study. Two patients in the treatment group tolerated $1,25(OH)_2D_3$ in the dose of 0.5 μg daily. In one patient (Pat. #3), serum calcium reached the upper limit of normal at the initial starting dose of 0.25 μg daily, and no increase in dosage was made. In four patients (Pats. #11, 15, 16, and 17), hypercalcemia occurred when the dose of $1,25(OH)_2D_3$ was increased to 0.5 μg daily. These episodes resolved within one week of stopping treatment, and these patients subsequently tolerated $1,25(OH)_2D_3$ at 0.25 μg daily with no further hypercalcemia. These episodes of hypercalcemia account for the rise in serum calcium in the treatment as compared with the control group, which was evident between 8 and 16 weeks (Fig. 1). This was preceded by or coincided with a rise in urinary calcium excretion (Fig. 2), rise in serum creatinine concentration (Fig. 3), and a fall in creatinine clearance (Fig. 4) in the treatment group. Serum calcium concentration was regularly higher in the treatment versus the control group throughout the study. However, this difference did not achieve statistical significance when analyzed by analysis of covariance. Urinary calcium excretion did not differ significantly when values throughout the study were analyzed. There was one episode of hypercalcemia (Pat. #14) in the placebo group.

Mean serum phosphorus rose in the placebo group, whereas, a small fall was registered in the treatment group (Fig. 5). This

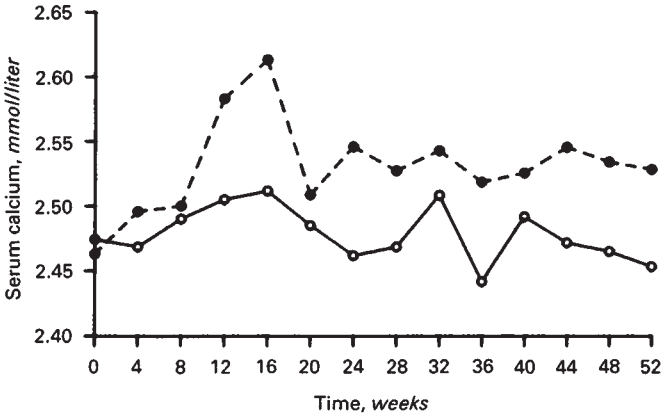

Fig. 1. *Serum calcium during 52 weeks of administration of $1,25(OH)_2D_3$ or placebo to patients with moderate renal failure.* Symbols are: (○----○) placebo; (●----●) $1,25(OH)_2D_3$.

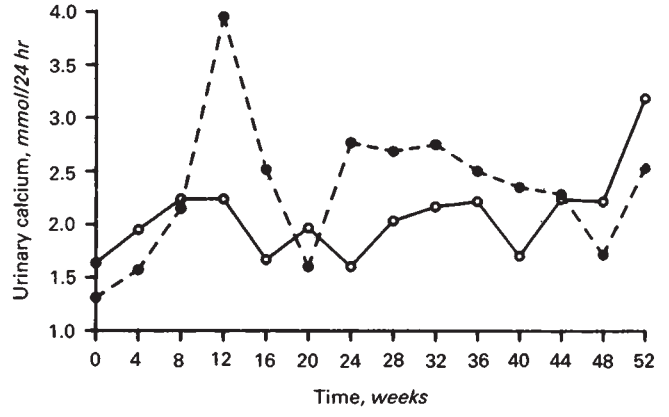

Fig. 2. *Urinary calcium during 52 weeks of administration of $1,25(OH)_2D_3$ or placebo to patients with moderate renal failure.* Symbols are: (○----○) placebo; (●----●) $1,25(OH)_2D_3$.

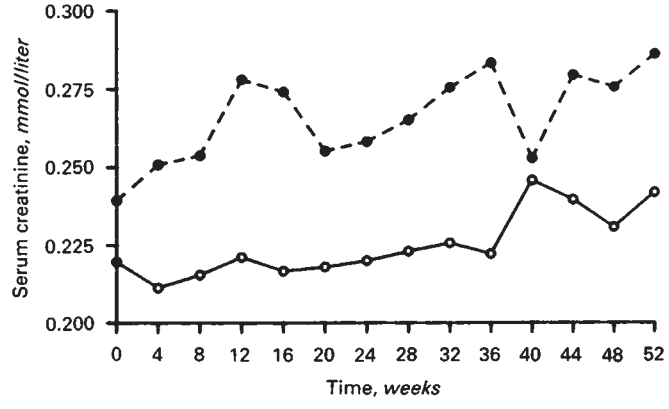

Fig. 3. *Serum creatinine during 52 weeks of administration of $1,25(OH)_2D_3$ or placebo to patients with moderate renal failure.* Symbols are: (○----○) placebo; (●----●) $1,25(OH)_2D_3$.

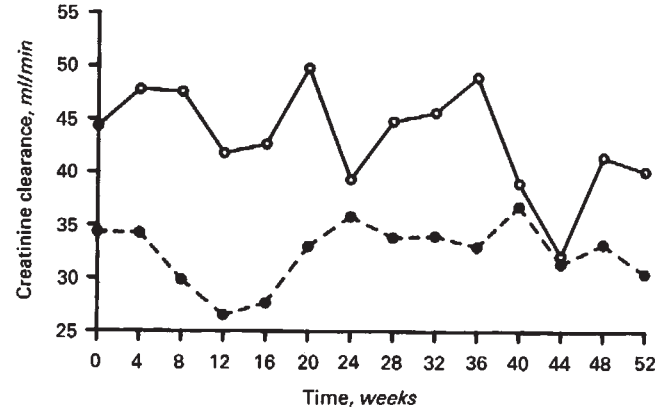

Fig. 4. *Creatinine clearance during 52 weeks of administration of $1,25(OH)_2D_3$ or placebo to patients with moderate renal failure.* Symbols are: (O----O) placebo; (●----●) $1,25(OH)_2D_3$.

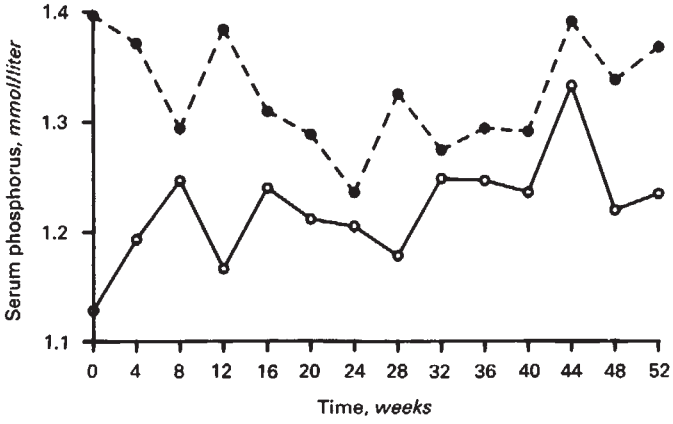

Fig. 5. *Serum phosphorus during 52 weeks of administration of $1,25(OH)_2D_3$ or placebo to patients with moderate renal failure.* Symbols are: (O----O) placebo; (●----●) $1,25(OH)_2D_3$.

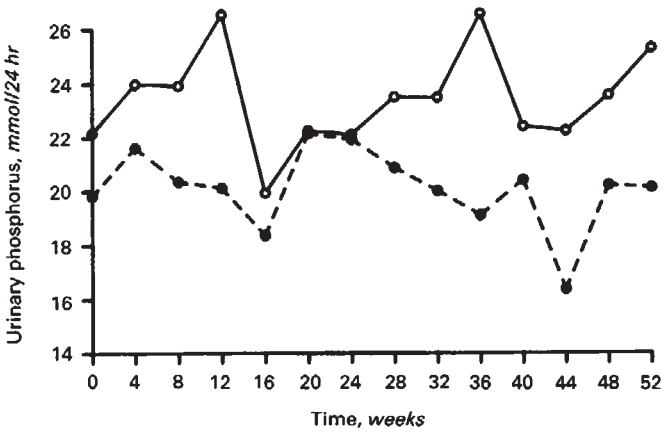

Fig. 6. *Urinary phosphorus during 52 weeks of administration of $1,25(OH)_2D_3$ or placebo to patients with moderate renal failure.* Symbols are: (O----O) placebo; (●----●) $1,25(OH)_2D_3$.

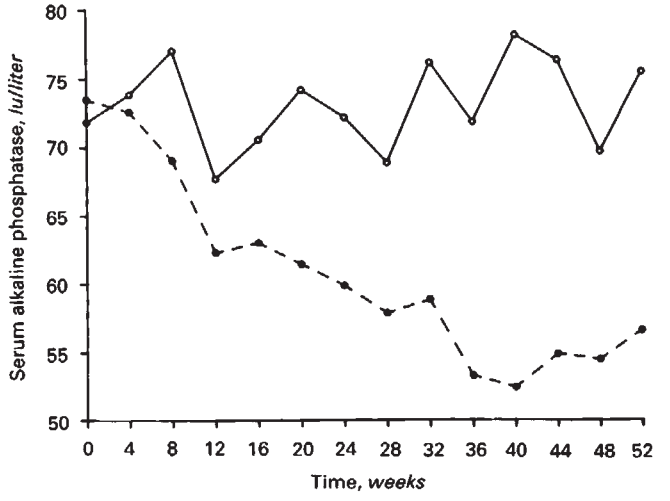

Fig. 7. *Serum alkaline phosphatase during 52 weeks of administration of $1,25(OH)_2D_3$ or placebo to patients with moderate renal failure.* Symbols are: (O----O) placebo; (●----●) $1,25(OH)_2D_3$.

independent effect of treatment on serum phosphorus concentration was highly significant ($P < 0.001$) and can still be demonstrated statistically when the patient who received an oral phosphate binder (Pat. #11; Alucaps, 2 tabs. t.i.d.) was excluded from the statistical analysis.

Urinary phosphorus was in the normal range in both treated and placebo patients at the beginning of the study, and there were no significant changes in either group during the study (Fig. 6).

There was a significant fall ($P < 0.01$), albeit within the normal range, of serum alkaline phosphatase concentration in treatment versus control patients (Fig. 7). Moreover, serum alkaline phosphatase levels at baseline correlated respectively with creatinine clearance ($r = -0.70$; $P < 0.01$), and parameters of osteoid and osteoblasts ($r = 0.52$ to 0.62; $P < 0.05$ to 0.01). In the treated group alkaline phosphatase levels no longer correlated with serum creatinine or creatinine clearance after therapy; however, the correlations with parameters of osteoid and osteoblasts were still observed ($r = 0.76$ to 0.62; $P =< 0.05$). At the beginning of the study, seven of the thirteen patients studied had elevated concentrations of parathyroid hormone. No change in mean parathyroid hormone concentration occurred in the placebo group during the study. A trend towards a fall in serum parathyroid hormone concentration was observed in the treatment group (Table 1); however, it did not reach statistical significance.

Mean 25-hydroxy D concentrations were in the low normal range in treatment and control groups before commencement of the study (Table 2). A rise of similar proportions in 25-hydroxy D concentrations was observed in each group during the study ($P < 0.05$).

The mean $1,25(OH)_2D$ concentrations were low in each group before commencement of the study, and no significant rise in trough $1,25(OH)_2D$ concentrations (blood samples taken immediately before the next morning dose) was observed. There was a positive correlation between serum $1,25(OH)_2D$ levels at baseline and at the end of the study ($r = 0.98$; $P < 0.001$) in the placebo group but not in the treatment group ($r = 0.29$).

During the course of the study there was a modest rise in serum creatinine concentration (Fig. 3) and a fall in creatinine

Table 2. Biochemical parameters in serum and urine of patients with moderate renal failure before and 12 months after therapy with 1,25(OH)$_2$D$_3$ placebo

Pt. no.	Serum calcium *mmol/liter*		Urinary calcium *mmol/24 hr*		Serum phosphorus *mmol/liter*		Urinary phosphorus *mmol/24 hrs*		Serum alkaline phosphatase *IU/ml*		Serum PTH *μg/ml*		Serum 25(OH)D *ng/ml*		1,25(OH)$_2$D *pg/ml*		Serum creatinine *mmol/liter*		Creatinine clearance *ml/min*	
Month	0	12	0	12	0	12	0	12	0	12	0	12	0	12	0	12	0	12	0	12
Placebo patients																				
6	2.39	2.31	4.1	4.4	1.04	1.11	24.9	31.1	61	61	0.5	0.4	17.0	36.0	21.2	32.0	.177	.177	46	39
8	2.54	2.44	1.2	0.8	1.03	1.04	26.1	30.4	76	69	0.4	0.4	5.1	16.2	15.6	16.7	.202	.177	53	47
10	2.50	2.57	2.5	0.7	1.39	1.81	27.0	24.4	96	86	0.9	1.0	11.1	35.0	4.4	3.2	.409	.569	27	23
12	2.55	2.46	1.2	0.8	1.06	1.05	25.4	28.8	61	72	0.5	0.6	11.4	27.2	4.8	8.7	.184	.194	56	50
13	2.47	2.47	0.6	0.8	1.14	1.30	15.6	13.3	98	108	0.8	0.7	15.8	27.7	26.4	37.9	.242	.235	30	24
14	2.41	2.47	1.3	5.5	1.05	1.10	14.1	24.0	42	57	0.9	0.5	50.5	47.0	23.6	35.0	.105	.100	56	58
Mean	2.48	2.45	1.65	2.17	1.12	1.24	22.2	25.3	72.3	75.5	0.67	0.60	18.5	31.5	16.0	22.2	.220	.242	44.7	40.2
±SD	±0.07	±0.08	±1.24	±2.19	±0.14	±0.3	±5.7	±6.6	±22.0	±18.8	±0.23	±0.23	±16.2	±10.4	±9.5	±14.6	±.103	±.166	±13.1	±14.3
1,25(OH)$_2$D$_3$ patients																				
1	2.46	2.55	0.5	1.3	1.34	1.30	12.3	12.7	67	67	1.0	0.8	18.2	28.4	14.8	21.7	.242	.239	25	27
3	2.50	2.59	1.2	2.0	1.27	1.64	21.0	25.4	94	44	0.9	1.0	13.3	25.6	19.2	14.0	.211	.316	27	24
5	2.47	2.56	0.4	3.6	1.32	1.44	23.5	27.2	45	37	0.3	0.3	18.5	30.4	14.4	21.7	.180	.196	59	59
11	2.35	2.54	0.9	3.1	1.85	1.36	25.1	17.5	124	87	1.6	0.6	46.0	48.6	17.7	17.6	.367	.469	20	14
15	2.50	2.43	1.6	2.6	1.27	1.23	20.6	27.3	72	66	0.9	0.7	27.8	36.5	11.8	16.6	.157	.168	41	37
16	2.46	2.48	3.2	4.5	1.06	1.00	25.6	28.2	61	57	0.4	0.4	24.0	31.4	18.0	16.3	.228	.239	45	44
17	2.52	2.55	0.8	0.7	1.68	1.61	10.7	9.8	53	38	1.0	0.6	13.0	38.6	16.0	15.6	.293	.378	26	15
Mean	2.47	2.53	1.23	2.54	1.40	1.37	19.8	21.2	73.7	56.6	0.87	0.63	23.0	34.2	16.0	17.6	.240	.286	34.7	31.4
±SD	±0.06	±0.05	±1.00	±1.32	±0.27	±0.22	±6.0	±7.7	±27.1	±18.3	±0.43	±0.24	±11.5	±7.8	±2.5	±3.0	±.071	±.108	±14	±16.3
Norm	<2.62		<7.5 male <6.25 female		<1.65		13–42		110		<0.9		18–35		17–54		0.06–0.12		120	

Statistical analysis using multiple regression analysis

clearance (Fig. 4) in each group, but there was no significant difference in the trends between groups in this respect. Creatinine clearance at baseline was lower in the treated than the control group. The difference was not statistically significant.

Highly significant correlations were found at commencement of the trial between: (a) creatinine clearance and both serum phosphorus ($P < 0.01$; $r = -0.70$) and parathyroid hormone ($P < 0.001$; $r = -0.78$); and (b) between parathyroid hormone and creatinine clearance ($r = -0.78$; $P < 0.001$), as well as serum phosphorus ($r = 0.76$; $P < 0.001$).

X-rays

The x-rays taken at the start, at six monthly intervals, and at the end of the study were not different and did not show clear-cut pathologic findings.

Bone histology

Histomorphometric parameters of bone structure, bone formation, resorption and bone dynamics are shown on Tables 3 through 5.

Parameters of bone structure

In all patients, cancellous bone mass, mean trabecular diameter, mean trabecular plate density and mean wall thickness were within the normal range at the beginning of the study and after 12 months of therapy. No significant changes were observed in these parameters (Table 4). At the beginning and end of the study none of the patients exhibited stainable aluminum at the bone osteoid interface or within bone.

Parameters of bone formation and resorption

Volume of lamellar osteoid was above the normal range at baseline in two out of the seven patients who received 1,25(OH)$_2$D$_3$ and in two of the controls receiving placebo (Table 3). Abnormal bone formation as evidenced by the presence of woven osteoid was found at baseline in all biopsies. Mean thickness of lamellar osteoid seams was not increased. The number of osteoblasts per unit of trabecular boundary length and the bone-osteoblast interface were elevated at baseline in eight and nine patients, respectively; therefore, the mean values for both of these histomorphometric parameters were significantly higher in the patients than in age- and sex-matched normal controls. Also, the number of osteoclasts per unit trabecular boundary length and the bone-osteoclast interface were elevated in nine and eleven patients, respectively, resulting in a significant increase in mean values of these parameters at baseline in both the patients that received placebo and 1,25(OH)$_2$D$_3$. Four patients exhibited trabecular fibrosis at baseline; those were the patients with the lowest creatinine clearances.

Administration of placebo for 12 months did not change lamellar osteoid volume and the number of osteoblasts and osteoclasts as well as bone-osteoblast interface and bone-osteoclast interface were unchanged and continued to be above the normal range. Volume and surface of woven osteoid showed a further increase.

Administration of 1,25(OH)$_2$D$_3$ for 12 months resulted in a significant decrease in lamellar osteoid volume and thickness. Woven osteoid volume and surface were significantly reduced

Table 3. Histomorphometric parameters of bone structure in patients with moderate renal failure before and 12 months after therapy with $1,25(OH)_2D_3$ or placebo

	$1,25(OH)_2D_3$ group		Placebo group		
	Before	After	Before	After	Normals[a]
Cancellous bone mass %	18.5 ± 0.9	17.1 ± 0.6	18.6 ± 1.9	21.8 ± 2.0	18.3 ± 0.7
Mean trabecular diameter *μm*	262 ± 10	270 ± 12	253 ± 9	280 ± 3	254 ± 4
Mean trabecular plate density *#/mm*	1.83 ± 0.13	1.64 ± 0.09	1.86 ± 0.15	1.74 ± 0.14	1.83 ± 0.05
Mean wall thickness *μm*	50.4 ± 1.7	51.2 ± 2.2	55.9 ± 2.7	60.7 ± 4.6	55.5 ± 1.2
Stainable aluminum at the bone-osteoid interface %	0	0	0	0	0

[a] Age and sex-matched normal controls

Table 4. Histomorphometric parameters of bone formation and resorption in patients with moderate renal failure before and 12 months after therapy with $1,25(OH)_2D_3$ or placebo

	$1,25(OH)_2D_3$ group		Placebo group		
	Before	After	Before	After	Normals[d]
Lamellar osteoid volume mm^3/cm^3	4.66 ± 1.07	2.21 ± 0.67[b,c]	6.27 ± 2.51	6.49 ± 1.66	4.05 ± 0.09
Lamellar osteoid surface %	15.6 ± 2.59	11.3 ± 3.10	19.6 ± 7.34	19.3 ± 4.72	15.4 ± 0.58
Mean osteoid seam thickness *μm*	9.47 ± 0.85	6.98 ± 0.44[b,c]	9.76 ± 0.90	10.70 ± 1.24	9.51 ± 0.18
Woven osteoid volume mm^3/cm^3	1.95 ± 0.70[a,b]	0.76 ± 0.60[a,c]	0.58 ± 0.16[a]	2.27 ± 0.65[a,c]	0
Woven osteoid surface %	4.20 ± 1.24[a]	2.06 ± 1.27[a,c]	1.56 ± 0.39[a]	5.04 ± 1.34[a,c]	0
Bone-osteoblast interface %	6.18 ± 0.82[a]	1.81 ± 0.28[b,c]	7.18 ± 2.45[a]	8.28 ± 1.84[a]	2.70 ± 0.20
Osteoblastic index *#/100 mm boundary length*	429 ± 58[a]	127 ± 17[b,c]	444 ± 144[a]	492 ± 118[a]	174 ± 11
Bone-osteoclast interface %	1.16 ± 0.16[a]	0.72 ± 0.26	1.64 ± 0.35	1.47 ± 0.35	0.73 ± 0.05
Osteoclastic index *#/100 mm boundary length*	42 ± 7.3[a]	24 ± 7.2	54 ± 13[a]	43 ± 10.6[a]	17 ± 0.91

[a] Differences between experimental groups and controls ($P < 0.05$)
[b] Differences between patients treated with $1,25(OH)_2D_3$ and placebo group ($P < 0.05$)
[c] Differences between baseline and 12 months of treatment or placebo within same group ($P < 0.05$)
[d] Age- and sex-matched normal controls

Table 5. Dynamic histomorphometric parameters of bone in patients with moderate renal failure before and 12 months after therapy with $1,25(OH)_2D_3$ or placebo

	$1,25(OH)_2D_3$ group		Placebo group		
	Before	After	Before	After	Normals[a]
Mineralization rate (*μm/day*)	0.59 ± 0.05	0.48 ± 0.08	0.47 ± 0.07	0.64 ± 0.07	0.51 ± 0.02
Doubly labelled trabecular surfaces %	3.01 ± .81	1.72 ± .41	2.6 ± 1.07	5.8 ± 1.26	3.76 ± 0.68
Mineralization lag time *days*	17.7 ± 3.70	16.4 ± 2.43	21.8 ± 2.18	17.5 ± 2.49	18.9 ± 0.78
Osteon remodelling time *days*	148 ± 34	187 ± 42	221 ± 45	158 ± 31	169 ± 2.2

[a] Age- and sex-matched normal controls

and parameters of bone cells showed a decrease in the number of osteoblasts and osteoclasts, that is, the osteoblastic index and osteoclastic index). At the end of $1,25(OH)_2D_3$ therapy only five out of seven patients still exhibited some woven osteoid, and the number of osteoblasts as well as bone-osteoblast interface was normal or low normal in all patients. The number of osteoclasts and bone-osteoclast interface was still above normal in three patients.

Dynamic parameters of bone

Mineral apposition rate was not significantly different from normal controls at baseline or after therapy in all patients (Table 5). Fraction of trabecular surfaces exhibiting double or single tetracycline labels was not different from normals at baseline. There was a trend towards a decrease in labelling after 12 months of treatment with $1,25(OH)_2D_3$ and a trend in the placebo group toward an increase in tetracycline labelling. Both trends did not achieve statistical significance.

Discussion

This is the first randomized prospective double-blind placebo-controlled trial in which the effects of $1,25(OH)_2D_3$ upon bone histology and serum biochemistry have been studied in patients with mild to moderate renal impairment. Published studies in patients with mild to moderate renal failure did not include an evaluation of the effects of the natural course of the progression of renal disease on bone [21, 22]. However, those data on mostly static parameters of bone histology are not substantially different from data reported here. The aim of the present study was to explore the efficacy and safety of $1,25(OH)_2D_3$ in such patients. Although patients were asymptomatic with normal serum alkaline phosphatase concentrations and normal bone x-rays, these studies cannot be described strictly as a trial of prophylaxis with $1,25(OH)_2D_3$ against the development of bone disease since baseline bone histology was abnormal. We have found evidence before treatment of an increased number of bone resorbing and forming cells in all patients, presence of woven osteoid in all patients, and fibrosis in some patients—findings that can be ascribed to increased parathyroid hormone activity on bone [18, 23]. The relationship of increased parathyroid hormone activity on bone to the renal impairment is documented by the statistically significant correlations of these histomorphometric parameters with creatinine clearance, serum creatinine, and serum phosphorus concentrations. Our failure to observe a statistically significant decrease in serum parathyroid hormone levels after $1,25(OH)_2D_3$ therapy might be related to the fact that a C-terminal assay was employed for measurement of serum parathyroid hormone concentrations. It is conceivable that this slight fall in creatinine clearance was associated with a reduction in clearance of PTH or C-terminal PTH fragments measured by the radioimmunoassay which could have blunted the ability of the assay to recognize an inhibition of PTH secretion during $1,25(OH)_2D_3$ administration.

The trend to a decrease in tetracycline labelling without a change in mineral apposition rate is also attributable to the lower bone turnover. The concomitant decrease in osteoid seam thickness and lack of change in mineralization lag time is evidence against any deterioration in mineralization induced by $1,25(OH)_2D_3$. The preferential fall in number of osteoblasts provides in vivo support for the in vitro finding of the presence of $1,25(OH)_2D_3$ receptors in osteoblasts and not in osteoclasts [24]. It is likely that with further therapy the number of osteoclasts as well as osteoblasts would decrease. This treatment-induced decrease in bone turnover is desirable only to the degree that the elevated turnover is brought back to normal, and low or below normal levels are not desirable. Whether alternative modes of administration (use of smaller doses of $1,25(OH)_2D_3$ or intermittent therapy) may ultimately avoid this potential for suppressing bone turnover to abnormally low levels is at present unknown and requires investigation.

Serum $1,25(OH)_2D$ concentrations were low or low normal at the commencement of the study and 24-hour trough serum $1,25(OH)_2D$ concentrations did not rise significantly on treatment. This observation was not unexpected, given the previous findings of Levine et al [25] that in normal humans serum levels of $1,25(OH)_2D$ are increased four hours but not 24 hours after oral administration of 0.5 μg $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ treatment was associated with significant effects upon serum alkaline phosphatase and bone histology, reflecting increased availability of $1,25(OH)_2D_3$.

Mean 25-hydroxy vitamin D_3 concentrations were low normal before treatment (Table 2) and rose to high normal levels in both placebo and $1,25(OH)_2D_3$ treated patients during the study. Dietary vitamin D intake in the United Kingdom may be borderline or low, and to exclude the possibility that this might influence results, both groups of patients received vitamin D_3, 400 units daily throughout the study. This accounts, in all probability, for the rise in $25(OH)D_3$ concentration observed.

A highly significant fall in serum alkaline phosphatase, albeit within the normal range, occurred in the $1,25(OH)_2D_3$ treated group. This is in keeping with previous observations in hemodialysis patients [2]. Siede, et al [26] found a correlation between bone alkaline phosphatase and osteoid as well as osteoblastic parameters in patients with mild to moderate renal failure. The same histologic abnormalities and correlations were found in the patients enrolled in this study. The percentage of total alkaline phosphatase of bone origin increased in patients with mild to moderate renal failure.

Our initial treatment protocol called for the dose of $1,25(OH)_2D_3$ to be increased to 0.5 μg daily. This proved to be too high a dose in most patients. As a result, a rise in urinary calcium followed or accompanied by hypercalcemia occurred in the earlier part of the study (Figs. 1, 2). This finding was associated with a rise in serum creatinine and a fall in creatinine clearance (Figs. 3, 4) and indicates that frequent monitoring of urinary calcium might be a valuable tool in the detection of impending hypercalcemia and prevention of loss of kidney function. An adjustment of dosage was followed by a decline in urinary calcium, correction of hypercalcemia and return of serum creatinine and creatinine clearance to pre-existing levels. Thereafter, serum calcium concentrations tended to be higher in the treatment than in the control group, although this did not reach statistical significance. A rising trend in serum phosphorus concentration occurred in the placebo group but not in the treatment group, and the difference between trends was statistically significant. This finding is in agreement with observations by Coen et al [27] who reported a fall in serum phosphorus concentration with $1,25(OH)_2D_3$ treatment. It is at variance with those of Christiansen et al [28], who noted no change in serum phosphorus.

Over the twelve months of the study, there was no significant deterioration in renal function attributable to treatment. This experience contrasts with that of Christiansen et al [13]. In their study, patients with a GFR of <35 ml/min were treated for six months with either 4000 IU of vitamin D or $1,25(OH)_2D_3$ at a starting dose of 1 μg/day. The majority of patients were unable to tolerate this high dose owing to hypercalcemia. Their pa-

tients differ from ours in having evidence of impaired calcium homeostasis. In each, at least two of the following were present: hypocalcemia; elevation of serum alkaline phosphatase; and reduced bone mineral content. One might have expected this group of patients to be less at risk of developing hypercalcemia in view of their "hungry bones", but we suspect that the relatively high dose of $1,25(OH)_2D_3$ used overcame this tendency. Alternatively, the possibility of aluminum accumulation in bone was not addressed, a possibility which could have rendered the patients prone to develop hypercalcemia. We found stainable aluminum in 5% of 100 unselected patients with a GFR between 20 and 66 ml/min (mean 36 ± 2.5 ml/min) [29]. Christiansen et al [13] suggested that $1,25(OH)_2D_3$ as formulated may itself be nephrotoxic irrespective of the presence of hypercalcemia. We found no evidence to support this claim.

Our patients had less severe impairment in renal function than those reported in previous studies [27, 28, 30, 31]. Since $1,25(OH)_2D_3$ is metabolized by the kidney our patients may have been exposed to lower concentrations of $1,25(OH)_2D_3$. The dose of $1,25(OH)_2D_3$ we chose was conservative, and we believe this to be the main explanation for the fact that we observed no deterioration in renal function attributable to treatment.

The long term aim of $1,25(OH)_2D_3$ treatment in patients with mild to moderate renal impairment is to protect bones against the consequences of progressive renal failure and to prevent parathyroid gland hyperplasia (with the subsequent need for parathyroid surgery) without affecting adversely renal function. We have demonstrated that these aims are at least in part realizable. Observation of much larger numbers of patients over a much longer period of time will be required to define whether the need for neck exploration can be reduced by $1,25(OH)_2D_3$ administration started when renal impairment is of mild to moderate degree. A previous study [2] provided no evidence of a reduction in the need for surgery in patients receiving $1,25(OH)_2D_3$ on regular hemodialysis. Treatment when end-stage renal failure has been reached may be too late to reduce the need for parathyroid surgery.

We are extremely nervous at the prospect that widespread administration of $1,25(OH)_2D_3$ to patients with mild to moderate renal impairment might be undertaken without careful follow-up and monitoring of urine and serum calcium and renal function. In 1980, Paterson [32] described twenty-seven episodes of hypercalcemia in twenty-one patients resulting from vitamin D poisoning. Two patients died. Failure of adequate follow-up was an important reason for such poisoning. We recommend that long-term $1,25(OH)_2D_3$ administration in patients such as those we described should be carried out only if frequent and meticulous follow-up can be ensured, and lower doses than those used in end-stage renal failure are employed.

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