Evidence for Abnormal Regulation of Circulating 1α,25-Dihydroxyvitamin D in Patients with Sarcoidosis and Normal Calcium Metabolism

PAULA H. STERN, JOSE DE OLAZABAL, and NORMAN H. BELL, *Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611; Veterans Administration Hospital and Departments of Medicine and Pharmacology, Medical University of South Carolina, Charleston, South Carolina 29403*

ABSTRACT The effects of vitamin D, 2.5 mg (100,000 U)/d for 4 d, on serum calcium, serum 25-hydroxyvitamin D (25-OHD), and serum 1α,25-dihydroxyvitamin D [1α,25$(OH)_2$D] were compared in 17 normal subjects and 6 patients with sarcoidosis who had normocalcemia and no history of hypercalcemia. The diagnosis was confirmed histologically in each of them. Vitamin D increased mean serum 25-OHD from 30±4 to 99±15 ng/ml ($P < 0.001$) and did not change mean serum 1α,25$(OH)_2$D (32±3 vs. 29±3 pg/ml) or mean serum calcium (9.5±0.1 vs. 9.6±0.1 mg/dl) in the normal subjects. In contrast, vitamin D increased mean serum 25-OHD from 19±3 to 65±19 ng/ml ($p < 0.05$), increased mean serum 1α,25$(OH)_2$D threefold from 40±7 to 120±24 pg/ml, and increased mean serum calcium from 9.4±0.2 to 9.8±0.2 mg/dl ($P < 0.01$). There was a significant positive correlation between the serum 1α,25$(OH)_2$D and serum calcium in these individuals ($r = 0.663$, $P < 0.01$) but not in the normal subjects. The results (*a*) provide further evidence for abnormal regulation of circulating 1α,25$(OH)_2$D in sarcoidosis and (*b*) indicate that the abnormality may exist in patients with normal calcium metabolism. Thus, the defect in vitamin D metabolism in sarcoid apparently is more common than was previously recognized.

INTRODUCTION

Available evidence indicates that the abnormal calcium metabolism in sarcoidosis results from increases in circulating 1α,25-dihydroxyvitamin D [1α,25$(OH)_2$D].[1] Mean plasma 1α,25$(OH)_2$D is increased and serum immunoreactive parathyroid hormone is suppressed in patients with sarcoidosis and hypercalcemia who do not have primary hyperparathyroidism (1, 2). Further vitamin D, 0.25 mg (10,000 U)/d for 12 d, increases serum 1α,25$(OH)_2$D abnormally in patients with normocalcemia and a history of hypercalcemia but does not alter serum 1α,25$(OH)_2$D abnormally in subjects or patients with sarcoid who have normal calcium metabolism (1). Thus, the increased sensitivity to vitamin D in sarcoid results from increases in serum 1α,25$(OH)_2$D. Finally, hypercalcemia and the increases in circulating 1α,25$(OH)_2$D are corrected after treatment with glucocorticoids (1, 2).

In our previous investigation this dose of vitamin D produced a modest but significant increase in mean serum 1α,25$(OH)_2$D (which remained well within the normal range) in the patients with sarcoidosis and normal calcium metabolism (1). To determine whether an abnormal response of serum 1α,25$(OH)_2$D might be elicited with a larger dose of vitamin D, the effects of 2.5 mg (100,000 U)/d for 4 d of the vitamin were compared in normal subjects and patients with sarcoidosis who had a normal serum calcium and had never had hypercalcemia.

METHODS

17 adult normal subjects, 9 men and 8 women ranging in age from 21 to 56 yr, and 6 patients with sarcoidosis, 4 men and 2 women ranging in age from 23 to 58 yr, were studied. They were hospitalized at the Clinical Research Center of the Indiana University Medical School or of the Medical University of South Carolina. Each of them was given a con-

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[1] *Abbreviations used in this paper*: 1α,25$(OH)_2$D, 1α,25-dihydroxyvitamin D; 25-OHD, 25-hydroxyvitamin D.

TABLE I
Clinical Findings in Patients with Sarcoidosis

Patient	Age	Sex	Serum Ca	Urinary Ca	Biopsy	Hypercalcemia*
	yr		*mg/dl*	*mg/d*		
A	58	F	9.0	112	+	−
B	27	M	9.4	—	+	−
C	33	F	9.2	174	+	−
D	26	M	9.5	255	+	−
E	31	M	9.6	86	+	−
F	23	M	9.6	156	+	−

* By history.

stant daily diet estimated to contain 400 mg/d of calcium and 900 mg of phosphorus and a constant fluid intake. Vitamin D_2, 2.5 mg (100,000 U)/d for 4 d, was given as a single morning dose. Fasting blood samples were obtained on the 1st d before vitamin D and again on the 5th d, 24 h after the last dose of the vitamin, for determination of calcium (3, 4), phosphorus (5), creatinine (6, 7), 25-hydroxyvitamin D (25-OHD), and 1α,25(OH)$_2$D. In the patients, 24-h urines were collected and analyzed for calcium (4).

Serum 25-OHD was measured in triplicate by the competitive protein-binding method with vitamin D-deficient rat serum (8) as described (1). The normal range for the method in this laboratory is from 10 to 80 ng/ml (1).

Serum 1α,25(OH)$_2$D was measured by bioassay as described (1, 9). The mean value in normal subjects is 32±2 pg/ml and the range is from 15 to 55 pg/ml (n = 33). The 95% confidence values (mean±2 SD) are 13–52 pg/ml. The interassay variation is 9.1% (n = 17).

Student's t test was used to determine the significance of differences between paired or unpaired samples (10). Correlation coefficient was carried out with a Hewlett-Packard calculator (model 9815A, Hewlett-Packard Co., Palo Alto, Calif.).

RESULTS

The diagnosis of sarcoidosis was confirmed histologically in each of the patients (Table I). None of them had had hypercalcemia and none had received steroids for at least 1 yr before the study. Serum calcium was normal in each of them and urinary calcium was above 200 mg/d in one of them.

The effects of vitamin D, 2.5 mg/d for 4 d, were examined in 17 normal subjects and in 6 patients with sarcoid (Table II). In the normal subjects vitamin D increased mean serum 25-OHD significantly, but did not alter mean serum calcium, phosphorus, creatinine, or 1α,25(OH)$_2$D.

In contrast, vitamin D increased mean serum calcium, phosphorus, 25-OHD, and 1α,25(OH)$_2$D and did not change mean serum creatinine in the patients. The increases in serum 1α,25(OH)$_2$D ranged from 57% in patient F to 797% in patient D and averaged 195%. Whereas before vitamin D mean serum 1α,25(OH)$_2$D was not significantly different between the normal subjects and patients, after vitamin D it was significantly higher in the patients than in the normal subjects ($P < 0.001$). Furthermore, there was a significant positive correlation between the serum 1α,25(OH)$_2$D and serum calcium in the patients (Fig. 1) but not in the normal subjects. Urinary calcium averaged 116, 139, 239, 72, and 214 mg/d

TABLE II
Effects of Vitamin D_2 on Serum Calcium, Phosphorus, Creatinine, 25-OHD, and 1α, 25(OH)$_2$D

	Calcium	Phosphorus	Serum creatinine	25-OHD*	1α,25(OH)$_2$D
	mg/dl	*mg/dl*	*mg/dl*	*ng/ml*	*pg/ml*
Normal subjects (17)					
Control	9.5±0.1	4.1±0.1	0.9±0.1	30±4	32±3
Vitamin D‡	9.6±0.1	4.1±0.1	1.0±0.1	99±15	29±3
P value	NS	NS	NS	<0.001	NS
Patients (6)					
Control	9.4±0.2	3.9±0.3	1.0±0.1	19±3	40±7
Vitamin D‡	9.8±0.2	4.2±0.3	1.0±0.1	65±19	120±24§
P value	<0.01	<0.01	NS	<0.05	NS

Results are given as mean±SE.

* Serum 25-OHD was measured in 12 normals before vitamin D and in 9 normals after vitamin D.

‡ Vitamin D_2, 2.5 mg/d for 4 d, was given by mouth.

§ The difference between control and treated values is not significant (t =2.05) because of the marked increase in serum 1α,25(OH)$_2$D from 34 to 305 pg/ml in patient D. If these values are omitted, mean serum 1α,25(OH)$_2$D increased from 42±8 to 83±13 pg/ml (t = 6.06, $P < 0.01$) in the other patients.

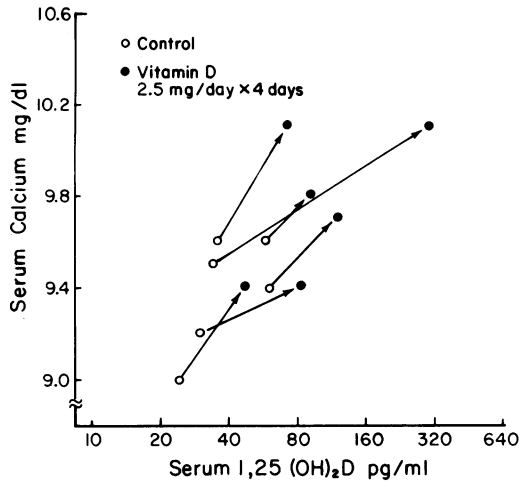

FIGURE 1 Effects of vitamin D_2 on serum $1\alpha,25(OH)_2D$ and serum calcium in six patients with sarcoidosis. Note that the serum $1\alpha,25(OH)_2D$ is drawn on a semi-log plot. $r = 0.663$; $P < 0.01$.

in five patients (A, C, D, E, and F, respectively) during the 4-d period of vitamin D administration and therefore changed very little (Table I).

DISCUSSION

Our studies show a clear impairment in the regulation of circulating $1\alpha,25(OH)_2D$ in a group of patients with sarcoid and apparent normal calcium metabolism. The results provide additional evidence that increases in serum $1\alpha,25(OH)_2D$ are responsible for the abnormal calcium metabolism in this disease (1, 2) and suggest that the defect in vitamin D metabolism is more common than was previously recognized.

The mechanism for the abnormal vitamin D metabolism in sarcoidosis has not been elucidated. Hypercalcemia was recently reported in a patient with sarcoid who had developed renal failure and had undergone bilateral nephrectomy in preparation for kidney transplantation (11). The hypercalcemia responded to steroids and was associated with suppression of serum immunoreactive parathyroid hormone and increases in serum $1\alpha,25(OH)_2D$. Serum calcium and serum $1\alpha,25(OH)_2D$ values correlated with each other. The findings in this patient support the possibility that in sarcoid the production of $1\alpha,25(OH)_2D$ may occur in tissues other than that of the kidney.

Our observations in normal subjects suggest that the renal production of $1\alpha,25(OH)_2D$ is tightly regulated. The findings are consistent with those obtained in patients with vitamin D intoxication, who show increases in serum 25-OHD and not $1\alpha,25(OH)_2D$ (12). In the rat, vitamin D intoxication with hypercalcemia is characterized by increases in serum 25-OHD and decreases in serum $1\alpha,25(OH)_2D$ (13). In contrast, vitamin D intoxication in the cow is characterized by increases in all of the metabolites of vitamin D including the vitamin itself, 25-OHD, 24,25-dihydroxyvitamin D, as well as $1\alpha,25(OH)_2D$ (14).

Urinary calcium did not change appreciably during the 4 d of treatment with vitamin D in our patients despite the increases in serum $1\alpha,25(OH)_2D$ and modest but significant increases in serum calcium, which remained within the normal range. We demonstrated previously in two patients with sarcoid and a history of hypercalcemia that vitamin D, 250 μg/d for 12 d, increased both serum $1\alpha,25(OH)_2D$ and urinary calcium. Serum calcium remained within the normal range. The increases in serum $1\alpha,25(OH)_2D$ were noted by the 5th d of administration of vitamin D and preceded the increases in urinary calcium. Urinary calcium did not change during the initial 4 d of vitamin D. Thus, the period of observation in the present study was too short an interval for an increase in urinary calcium to occur.

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