

Effects of Active Vitamin D₃ and Parathyroid Hormone on the Serum Osteocalcin in Idiopathic Hypoparathyroidism and Pseudohypoparathyroidism

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Abstract

Serum osteocalcin was measured in patients with idiopathic hypoparathyroidism or pseudohypoparathyroidism, before or during the treatment with active vitamin D₃ (1,25(OH)₂D₃ or 1αOHD₃).

Serum osteocalcin and plasma 1,25(OH)₂D were decreased in 11 patients with idiopathic hypoparathyroidism before treatment (2.8 ± 1.27 ng/ml, $P < 0.001$ and 14.3 ± 4.27 pg/ml, $P < 0.001$, respectively). In 24 patients with idiopathic hypoparathyroidism during the treatment, serum osteocalcin and plasma 1,25(OH)₂D were within the normal range (4.5 ± 0.74 ng/ml and 25.7 ± 5.69 pg/ml, respectively).

In five patients with pseudohypoparathyroidism before treatment, plasma 1,25(OH)₂D was decreased (15.6 ± 10.6 pg/ml, $P < 0.001$) but serum osteocalcin was normal (7.8 ± 1.66 ng/ml). In nine patients with pseudohypoparathyroidism during the treatment with active vitamin D₃, serum osteocalcin and plasma 1,25(OH)₂D were normal (6.8 ± 1.47 ng/ml and 27.2 ± 6.0 pg/ml, respectively). Serum PTH in pseudohypoparathyroidism was increased before treatment (0.70 ± 0.34 ng/ml, $P < 0.05$) and was normal during the treatment (0.50 ± 0.13 ng/ml).

In idiopathic hypoparathyroidism, the active vitamin D₃ increased serum osteocalcin without PTH. In pseudohypoparathyroidism, PTH may increase serum osteocalcin or modulate the effect of active vitamin D₃ on serum osteocalcin.

Introduction

Osteocalcin, also called γ-carboxyglutamic acid-containing protein of bone (1, 2), is presumed to be produced by osteoblasts (3, 4), and circulating osteocalcin is believed to be derived from new cellular synthesis (5). Serum levels of osteocalcin can be measured by RIA (6) and may provide a sensitive biochemical marker for bone turnover (7–10), especially for bone formation (11–13). In vitro studies demonstrate that osteoblasts contain receptors of the active vitamin D metabolite 1,25(OH)₂D (14) and osteocalcin synthesis in osteoblast-like osteosarcoma cell culture is stimulated by 1,25(OH)₂D₃ (15). An increase in serum osteocalcin after 1,25(OH)₂D treatment was reported in patients with inherited rickets (16), postmenopausal osteoporosis (17), and in normal subjects (18).

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In idiopathic hypoparathyroidism and pseudohypoparathyroidism, plasma 1,25(OH)₂D is low (19–21) due to the lack of PTH or to the unresponsiveness of the kidney to PTH (22–24), and active vitamin D₃ (1,25(OH)₂D₃ or 1αOHD₃) are administered (25–28) as the treatment. Several reports proved that serum osteocalcin is low in hypoparathyroidism (7, 29). To investigate the effect of active vitamin D₃ on serum osteocalcin in idiopathic hypoparathyroidism, we compared serum osteocalcin levels in patients with idiopathic hypoparathyroidism before and during the treatment with active vitamin D₃. Serum osteocalcin is increased in primary hyperparathyroidism (7, 30, 31), but osteocalcin secretion by bone was decreased by PTH treatment in vitro and by infusion in vivo (32, 33). Serum immunoreactive PTH is low or undetectable in idiopathic hypoparathyroidism but is increased in pseudohypoparathyroidism. In some patients with pseudohypoparathyroidism, bone is thought to be fully responsive to PTH even though the renal responses may be deficient (34, 35). To clarify the effect of PTH on serum osteocalcin, we compared serum osteocalcin levels in idiopathic hypoparathyroidism with those in pseudohypoparathyroidism.

Methods

Subjects. We studied 11 patients with idiopathic hypoparathyroidism before treatment (6 men and 5 women, mean age 43 ± 14.3 yr), and 25 with idiopathic hypoparathyroidism during treatment with active vitamin D₃ (17 men and 8 women, mean age 46 ± 14.9 yr). Of the latter patients, 12 were administered 1.7 ± 0.75 μg/d of 1,25(OH)₂D₃ and 13 were administered 3.8 ± 0.87 μg/d of 1αOHD₃. In seven patients, serum osteocalcin was measured before and during treatment.

We also studied 5 patients with pseudohypoparathyroidism before treatment (3 men and 2 women, mean age 35 ± 13.5 yr), and 9 with pseudohypoparathyroidism during the treatment with active vitamin D₃ (5 men and 4 women, mean age 36 ± 12.5 yr). Of the latter patients, 5 were administered 1.1 ± 0.22 μg/d of 1,25(OH)₂D₃ and 4 were administered 1.8 ± 0.50 μg/d of 1αOHD₃. In four patients, serum osteocalcin was measured before and during treatment.

The diagnosis of idiopathic hypoparathyroidism or pseudohypoparathyroidism was established by clinical symptoms along with hypocalcemia, hyperphosphatemia, low values of nephrogenous cAMP, serum levels of immunoreactive PTH, and urinary phosphate and cAMP responses to exogenous PTH (36).

The doses of active vitamin D₃ that would maintain the serum calcium in the normal range (8.4–10.2 mg/dl) were determined. The maintenance dose of 1,25(OH)₂D₃ in pseudohypoparathyroidism was not significantly lower than that in idiopathic hypoparathyroidism, but the optimal maintenance dose of 1αOHD₃ in pseudohypoparathyroidism was significantly lower than that in idiopathic hypoparathyroidism (3.8 ± 0.87 vs. 1.8 ± 0.50 μg/d, $P < 0.001$).

The duration of treatment after serum calcium reached the normal range was 4.7 ± 3.53 (mean \pm SD) yr (range, 0.5–10.5 yr) in 25 patients with idiopathic hypoparathyroidism and 4.5 ± 3.47 yr (range, 0.8–10 yr) in 9 patients with pseudohypoparathyroidism.

We also studied 68 normal controls, 36 men and 32 women, with a mean age of 39 ± 16.5 yr. The normal subjects had no evidence of

Table I. Descriptive Characteristics and Biochemical Data of Experimental Subjects

	Idiopathic hypoparathyroidism		Pseudohypoparathyroidism		Normal controls
	Before treatment	During treatment	Before treatment	During treatment	
No. (male/female)	11 (6:5)	25 (17:8)	5 (3:2)	9 (5:4)	68 (36:32)
Age (yr)	43±14.3	46±14.9	35±13.5	36±12.5	39±16.5
Serum Ca (mg/dl)	6.5±1.22*	8.8±0.34	6.9±1.19*	8.9±0.37‡	9.3±0.45
Serum P (mg/dl)	5.4±0.81	4.1±0.49	5.4±0.96	3.9±0.29‡	3.6±0.40
Serum osteocalcin (ng/ml)	2.8±1.27*	4.5±0.74* §	7.8±1.66§	6.8±1.47	6.2±1.93
Serum ALP (IU/ml)	73±21.1	78±19.9	63±32.2	66±24.9	80±25.0
Plasma 1,25(OH) ₂ D (pg/ml)	14.3±4.27*	25.7±0.74§	15.6±10.6*	27.2±6.0‡	39.7±10.8
GFR (ml/min per 1.48 m ²)	83±24.7	75±17.4	83±12.9	81±15.4	100±10.0
Serum PTH (ng/ml)	0.10±0.05 [†]	0.08±0.02	0.70±0.34**	0.43±0.24	0.50±0.13
Dose of vitamin D (μg/d)					
1,25(OH) ₂ D ₃ (n)		1.7±0.75 (12)		1.1±0.22 (5)	
1αOHD ₃ (n)		3.8±0.87 (13)		1.8±0.50 (4)	

* $P < 0.001$, vs. normal controls. ‡ $P < 0.001$, vs. pseudohypoparathyroidism before treatment. § $P < 0.001$, vs. idiopathic hypoparathyroidism before treatment. ^{||} $P < 0.001$, vs. idiopathic hypoparathyroidism during treatment. [†] Values lower than detection limit were estimated as 0.075 ng/ml. ** $P < 0.05$, vs. normal controls.

calcium or skeletal abnormalities by routine history, physical examination, and biochemical evaluation. All subjects and patients were studied after informed consent was obtained.

Procedures. For all subjects and patients, serum and heparinized plasma were collected between 0800 and 1000 hours for biochemical determinations after an overnight fast.

Serum osteocalcin concentrations were determined by RIA with a Midori Fuji RIA kit (CIS; Compagnie Oris Industrie Société Anonyme, Saclay, France). This assay used a rabbit anti-bovine osteocalcin antibody and bovine osteocalcin as a standard and tracer. After a 24-h nonequilibrium incubation, antibody-bound and free osteocalcin were separated by the double antibody method using a complex of sheep anti-rabbit IgG and polyethylene glycol. The sensitivity of the assay was 0.35 ng/ml. The intra- and interassay variations were < 3.6 and 6.8%, respectively. All standards and samples were analyzed in duplicate.

Plasma 1,25(OH)₂D was measured by radioreceptor assay, as previously described (37). Serum immunoreactive PTH was determined using an RIA kit (Yamasa Shouyu Co., Choshi, Japan). This assay used a chicken anti-human PTH (43–68) antibody. The sensitivity of this assay was 0.075 ng/ml and the intra- and interassay variations were less than 2.7 and 5.3%, respectively. Serum total alkaline phosphatase (ALP) was measured by an automated technique. Serum calcium and phosphate were measured by EGTA titrimetry, and Fiske and Subbarow's method (38), respectively. Glomerular filtration rate was assessed by creatinine clearance. Serum and urine creatinine were measured by Brod and Sirotta's method (39).

Differences between groups were analyzed by the unpaired t test. Correlations between the biochemical measurements were assessed by using linear regression analysis.

Results

The results are presented in Table I and in Figs. 1–3. In idiopathic hypoparathyroidism before treatment, serum osteocalcin and plasma 1,25(OH)₂D levels were lower than those in normal subjects (2.8 ± 1.27 vs. 6.2 ± 1.93 ng/ml, $P < 0.001$; and 14.3 ± 4.27 vs. 39.7 ± 10.8 pg/ml, $P < 0.001$, respectively).

In idiopathic hypoparathyroidism, during the treatment with active vitamin D₃, serum osteocalcin and plasma 1,25(OH)₂D levels were higher than those in the patients before treatment (2.8 ± 1.27 vs. 4.5 ± 0.74 ng/ml, $P < 0.001$; and 14.3 ± 4.27 vs. 25.7 ± 5.69 pg/ml, $P < 0.001$, respectively). The level of serum osteocalcin during treatment was higher than that before treatment in all 7 patients studied before and during treatment. But serum osteocalcin was lower in 25 patients during treatment than in the normal controls (4.5 ± 0.74 vs. 6.2 ± 1.93 ng/ml, $P < 0.001$). In idiopathic hypoparathyroidism before or during the treatment, there was no significant correlation between serum osteocalcin and plasma 1,25(OH)₂D.

In pseudohypoparathyroidism before treatment, the plasma 1,25(OH)₂D level was lower than that in normal controls (15.6 ± 10.6 vs. 39.7 ± 10.8 pg/ml, $P < 0.001$) and was not significantly different from the values found in the patients with idiopathic hypoparathyroidism before treatment. But serum osteocalcin was higher compared with the patients with idiopathic hypoparathyroidism before treatment (7.8 ± 1.66 vs. 2.8 ± 1.27 ng/ml, $P < 0.001$), and was not significantly different from the value in normal controls.

In pseudohypoparathyroidism during the treatment, plasma 1,25(OH)₂D was not significantly different from the value in idiopathic hypoparathyroidism during the treatment,

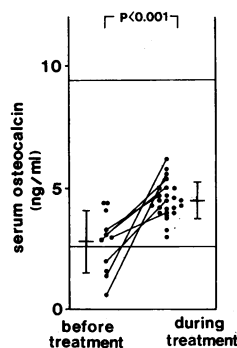


Figure 1. Serum osteocalcin in patients with idiopathic hypoparathyroidism before or during the treatment with active vitamin D₃. Serum osteocalcin was significantly lower in cases before treatment than in cases during the treatment. In seven cases, serum osteocalcin was measured both before and during the treatment. Serum osteocalcin was higher during treatment than before in all of these seven cases.

1. Abbreviations used in this paper: ALP, alkaline phosphatase.

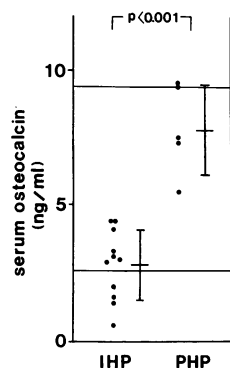


Figure 2. Serum osteocalcin in patients with idiopathic hypoparathyroidism (IHP) or pseudohypoparathyroidism (PHP) before treatment. Serum osteocalcin was significantly higher in cases with pseudohypoparathyroidism than that in cases with idiopathic hypoparathyroidism.

but the serum osteocalcin value was higher than that in idiopathic hypoparathyroidism (6.8 ± 1.47 vs. 4.5 ± 0.74 ng/ml, $P < 0.001$). The serum osteocalcin value in pseudohypoparathyroidism during treatment was not significantly different from that in pseudohypoparathyroidism before treatment. There was no significant difference in serum osteocalcin before and during treatment in four patients with pseudohypoparathyroidism studied before and during treatment, either.

Serum total ALP was not significantly different among the four groups of patients and normal controls, and there was no significant correlation between serum osteocalcin and serum total ALP.

Serum Ca and P concentrations in idiopathic hypoparathyroidism was not significantly different from those in pseudohypoparathyroidism either before or during the treatment. In idiopathic hypoparathyroidism before or during treatment, the coefficients of the correlation between serum Ca and serum osteocalcin, or between serum phosphate and serum osteocalcin were 0.459 ($P < 0.01$) and -0.515 ($P < 0.01$), respectively.

Serum PTH was increased in pseudohypoparathyroidism before treatment (0.70 ± 0.34 , vs. normal 0.5 ± 0.13 ng/ml, $P < 0.05$). In pseudohypoparathyroidism during treatment, serum PTH was not affected significantly but it was lower than in pseudohypoparathyroidism before treatment.

Glomerular filtration rates were not different among the four groups of patients.

Discussion

Osteocalcin is a unique osteoblastic product (3, 4) and serum osteocalcin is a sensitive biochemical marker of bone turnover (7–10). Active vitamin D metabolite $1,25(\text{OH})_2\text{D}$ stimulates osteocalcin synthesis in vitro (15) and increases serum osteo-

calcin in vivo (16–18). In hypoparathyroidism, turnover of calcium is decreased in kinetic studies with labeled calcium (40) and in which serum osteocalcin is low (7, 29). Plasma $1,25(\text{OH})_2\text{D}$ is also low in hypoparathyroidism before treatment (19, 20, 22). In the present study, serum osteocalcin and plasma $1,25(\text{OH})_2\text{D}$ levels were significantly decreased in idiopathic hypoparathyroidism before treatment, and were significantly higher in cases during the treatment with $1,25(\text{OH})_2\text{D}_3$ or $1\alpha\text{OHD}_3$ than those in cases before treatment. These data were consistent with previous reports (7, 15–18, 29) and indicated that $1,25(\text{OH})_2\text{D}_3$ and $1\alpha\text{OHD}_3$ (probably after 25-hydroxylation in the liver) increase circulating levels of osteocalcin in idiopathic hypoparathyroidism without PTH. But there is an important question. Though supraphysiological doses (28, 41) of active vitamin D_3 were administered to patients with idiopathic hypoparathyroidism, serum osteocalcin in idiopathic hypoparathyroidism during the treatment was lower than in the normal controls. This may be due to lack of PTH in idiopathic hypoparathyroidism.

There is disagreement about the effect of PTH on osteocalcin synthesis and/or secretion. Serum osteocalcin is increased and is correlated with the serum levels of PTH in primary hyperparathyroidism (31). But increased serum osteocalcin may not be solely due to increased serum PTH because circulating levels of $1,25(\text{OH})_2\text{D}$ are also generally high in primary hyperparathyroidism (42). Several reports indicated the inhibitory effect of PTH on osteocalcin secretion by bone (32, 33). It is important to separate the action of PTH and $1,25(\text{OH})_2\text{D}_3$ on osteocalcin secretion in order to establish the effect of PTH. In pseudohypoparathyroidism, serum PTH is increased (43) but plasma $1,25(\text{OH})_2\text{D}$ is low due to deficient responsiveness of kidney to PTH (23, 24). Bone is thought to be fully responsive to PTH (34, 35), in some cases with pseudohypoparathyroidism. We measured serum osteocalcin in pseudohypoparathyroidism to clarify the effect of PTH on serum osteocalcin and the responsiveness of bone to PTH in pseudohypoparathyroidism. Plasma $1,25(\text{OH})_2\text{D}$ levels in pseudohypoparathyroidism before treatment and in idiopathic hypoparathyroidism before treatment were similarly low, but serum osteocalcin in the former was within the normal range and was significantly higher than that in the latter. The optimal maintenance dose or minimum daily dose of active vitamin D_3 was lower in pseudohypoparathyroidism than in idiopathic hypoparathyroidism, but serum osteocalcin was higher in pseudohypoparathyroidism during treatment than in idiopathic hypoparathyroidism during treatment. These results suggest that PTH may increase serum osteocalcin with or without a small amount of $1,25(\text{OH})_2\text{D}$ in pseudohypoparathyroidism before treatment, and that PTH may modulate the effect of $1,25(\text{OH})_2\text{D}_3$ on circulating levels of osteocalcin in pseudohypoparathyroidism during treatment with active vitamin D_3 . PTH concentration seems to be the important determinant of serum osteocalcin in pseudohypoparathyroidism and probably is such in primary hyperthyroidism as well.

In pseudohypoparathyroidism during treatment with active vitamin D_3 , plasma $1,25(\text{OH})_2\text{D}$ was higher than it was in pseudohypoparathyroidism before treatment, but the serum osteocalcin level was not significantly different from that in the latter. This may be due to the decrease of circulating PTH. But it has been reported that $1,25(\text{OH})_2\text{D}_3$ may decrease the sensitivity of bone cells to PTH by decreasing guanine nucleotide regulatory subunits (44, 45). Further studies are needed to

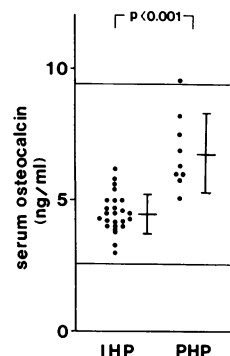


Figure 3. Serum osteocalcin in patients with idiopathic hypoparathyroidism (IHP) or pseudohypoparathyroidism (PHP) during the treatment with active vitamin D_3 . Serum osteocalcin in cases with pseudohypoparathyroidism was significantly higher than in cases with idiopathic hypoparathyroidism.

decide whether $1,25(\text{OH})_2\text{D}_3$ modulates the effect of PTH on osteocalcin synthesis, especially in pseudohypoparathyroidism.

In conclusion, our data showed that active vitamin D_3 , $1,25(\text{OH})_2\text{D}_3$ and $1\alpha\text{OHD}_3$ (probably after 25-hydroxylation in the liver), increased serum osteocalcin in idiopathic hypoparathyroidism without PTH, and that PTH may increase serum osteocalcin and may modulate the effect of active vitamin D_3 on serum osteocalcin in pseudohypoparathyroidism.

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