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Effect of 1,25(OH)$_2$ Vitamin D$_3$ on Circulating Insulin-Like Growth Factor-I and β_2 Microglobulin in Patients with Osteoporosis

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Abstract. To test the hypothesis that growth factors mediate the stimulatory effect of 1,25(OH)$_2$vitamin D$_3$ [1,25(OH)$_2$D$_3$] on bone remodeling in osteoporosis, the authors studied the effect of the secosteroid administration in two doses (1 μg/day and 2 μg/day) for 14 days on circulating insulin-like growth factor-I (IGF-I), β_2 microglobulin, and osteocalcin in 18 osteoporotic women. The biological effectiveness of the treatment was controlled by a decline of serum intact parathyroid hormone. Compared with the values before treatment, 1,25(OH)$_2$D$_3$ increased means of plasma IGF-I, β_2 microglobulin, and serum osteocalcin significantly; however, the effects were only apparent after the higher dose of the drug (169 ± 26 versus 134 ± 28 ng/ml, $P < 0.01$; 2.08 ± 0.1 versus 1.92 ± 0.1 μg/ml, $P < 0.05$; and 8.5 ± 1.3 versus 5.4 ± 1.1 ng/ml, $P < 0.01$, respectively). The authors conclude that exogenous 1,25(OH)$_2$D$_3$ promotes the production of IGF-I and β_2 microglobulin in osteoporotic patients in parallel to the marker of osteoblastic function, osteocalcin, which supports the tested hypothesis.

Key words: Insulin-like growth factor-I — β_2 microglobulin — Osteocalcin — 1,25(OH)$_2$vitamin D$_3$.

Insulin-like growth factor-I (IGF-I) is synthetized by multiple tissues, predominantly the liver and bone [1–4]. The peptide is believed to be one of the most prevalent autocrine/paracrine activators of osteoblast function [1, 5–7]. It is proposed that IGF-I mediates the anabolic effects of important skeletal hormones such as parathyroid hormone (PTH), androgens, and estrogen [8–13]. Another bone-derived growth factor is β_2 microglobulin [14]. However, data on the effect of 1,25(OH)$_2$D$_3$ on the production of the latter peptide in patients with osteoporosis are missing, although the vitamin is used in the treatment of the disease. Bone remodeling is effectively stimulated also by 1,25(OH)$_2$D$_3$ [8, 15]. The action of the secosteroid might be direct, through the specific receptors on osteoblasts [8], as well as mediated by growth factors [16].

The objective of the study was to determine whether exogenous 1,25(OH)$_2$D$_3$ influences IGF-I and β_2 microglobulin in plasma of osteoporotic patients.

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Materials and Methods

Subjects

Eighteen otherwise healthy white European osteoporotic women participated in the study. All had been without medication for at least 1 month and had never been treated with bisphosphonates, calciferol, or estrogen. None had compressive fractures. Osteoporosis was diagnosed by dual energy X-ray densitometry (DXA, Hologic VQDR-2000, USA, mean Z score of the axial skeleton in L_1–L_4 region was −1.46 ± 0.33 and of the hip −1.54 ± 0.22). The women were divided into two groups. The first group contained nine women (two with senile osteoporosis and the remainder with postmenopausal) treated with 1,25(OH)$_2$D$_3$ (Rocaltrol, F. Hoffman La-Roche, Switzerland) in doses of 1 μg/day; mean ages 56.0 ± 2.3 (±SEM). The second group also comprised nine women (two with senile osteoporosis, the remainder with postmenopausal), to whom 2 μg/day of the secosteroid was prescribed; mean ages 58.3 ± 3.0 (±SEM). All of the subjects were fully informed as to the purpose of the study and gave their written consent. The protocol was approved by the local Ethical Committee of the Institute of Endocrinology in Prague.

Protocol

Blood samples for the estimation of IGF-I, β_2 microglobulin, osteocalcin, intact PTH, 25OHD$_3$ and total calcium were collected after fasting in the morning. The same procedure was followed once more after the oral administration of 1,25(OH)$_2$D$_3$ for 14 days.

Laboratory Methods

Plasma IGF-I concentrations were assessed by a commercial radioimmunoassay (RIA) kit (Amersham, England). Plasma β_2 microglobulin levels were also estimated by an RIA method (IMMUNOTECH, Czech Republic). Immunoradiometric estimations were provided for PTH and osteocalcin (Allégro Intact PTH and Human Osteocalcin Kit also from the Nichols Institute, USA). Serum 25OHD$_3$ was assessed after extraction radioimmunologically using a 25OHD$_3$ 60T kit from the Nichols Institute. Serum calcium was measured automatically on Merck VitaLab by a kit from Merch, Germany. Duplicate determinations were used to form mean values. All hormonal specimens from each given participant were included in the same assay. The intraassay coefficients of variation were 5% for IGF-I, 3.2% for β_2 microglobulin, 4.7% for osteocalcin, 3.0% for PTH, 3.5% for 25OHD$_3$, and 4.9% for calcium. The interassay coefficient of variation for calcium was 5.4%.

Calculation and Statistics

The results presented are means ± SEM. Because data were found

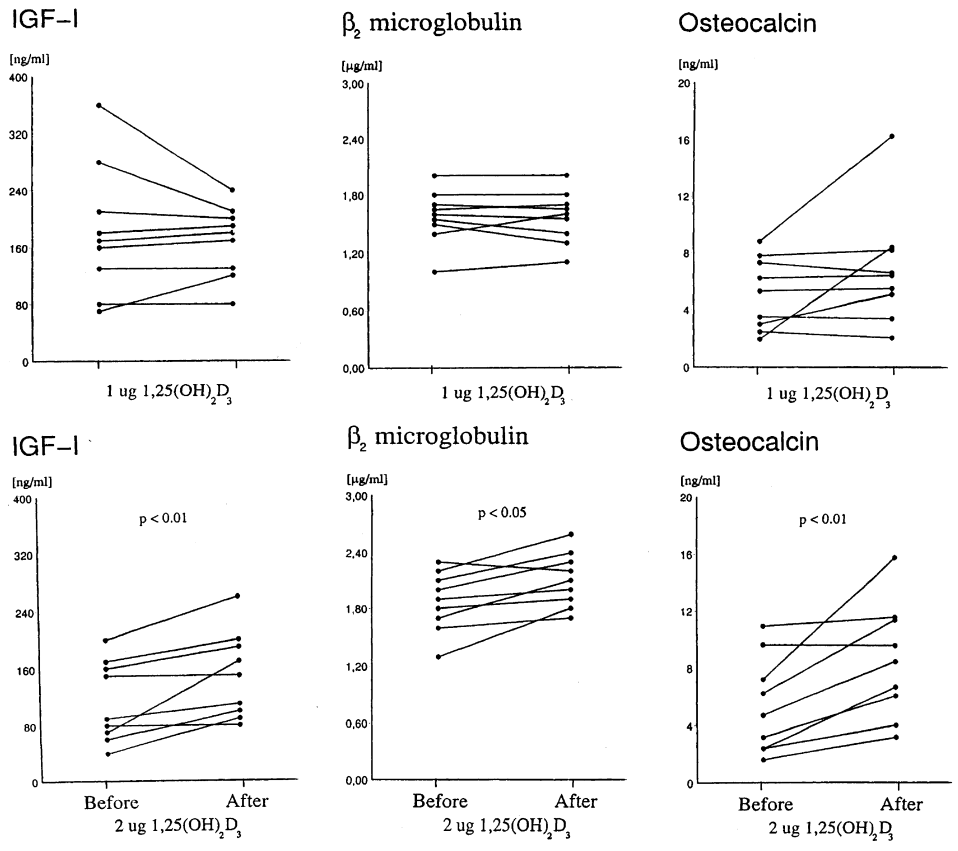

Fig. 1. Plasma IGF-I, β_2 microglobulin, and serum osteocalcin levels of individual osteoporotic women before and after 1 μg or 2 μg/day of $1,25(OH)_2D_3$.

to be normally distributed, the Student's paired two-tailed *t*-test was used to compare the values of the investigated parameters before and after treatment. Student's unpaired *t*-test was used to compare the initial values of the investigated parameters between both groups.

Results

Plasma IGF-I

After 1 μg/day of $1,25(OH)_2D_3$, IGF-I levels increased in three patients, decreased in another three, and were unchanged in the remaining women (Fig. 1). Mean values of the parameter before and after treatment did not differ significantly (Table 1). The 2 μg/day dose of the secosteroid elevated IGF-I concentrations in all of the investigated women (Fig. 1). An increase in means of peptide levels was noted ($P < 0.01$ as compared with the values before the treatment; Table 1).

Plasma β_2 Microglobulin

After 1 μg/day of $1,25(OH)_2D_3$, β_2 microglobulin concentrations increased in four women, decreased in another three, and remained the same in two (Fig. 1). Mean values of the peptide after treatment were not altered by the administration of the secosteroid (Table 1). A two times higher dose of the vitamin stimulated β_2 microglobulin levels in eight patients and decreased them in one woman (Fig. 1). Mean values of the peptide were higher after $1,25(OH)_2D_3$ administration ($P < 0.05$ compared with the values before the treatment; Table 1).

Serum Osteocalcin

Although an increasing tendency was documented in osteocalcin levels after treatment with 1 μg/day of $1,25(OH)_2D_3$, significant elevation was documented only after the higher dose in all but one patient ($P < 0.01$ as compared with mean values before treatment, Table 1, Fig. 1).

Serum PTH, $25OHD_3$, and Calcium

A decline in PTH concentrations was apparent in all investigated women after both the higher and lower sterol doses ($P < 0.01$ for means after both doses of $1,25(OH)_2D_3$ compared with the values before treatment). Mean serum $25OHD_3$ was of low normal value (Table 1), which was a result of subnormal levels of the parameter in three patients from each group. Means of serum calcium levels after both doses of the secosteroid were elevated, however, significantly only after the higher dose ($P < 0.05$ as compared with the values before the treatment) (Table 1).

No differences were observed in the initial values of all of the investigated parameters between both groups.

Discussion

The present study revealed that the systemic administration

Table 1. Mean values of the parameter before and after treatment with $1,25(OH)_2D_3$*

		$(1,25(OH)_2D_3)$ 1 μg/day		$(1,25(OH)_2D_3)$ 2 μg/day	
	Normal range	Before	After	Before	After
IGF-I ng/ml	20–200	181 ± 32	167 ± 17	134 ± 28	169 ± 26[a]
β_2 Microglobulin μg/ml	1.0–2.4	1.57 ± 0.09	1.57 ± 0.08	1.92 ± 0.1	2.08 ± 0.1[b]
Osteocalcin ng/ml	2.4–11.7	5.16 ± 0.84	6.89 ± 1.36	5.36 ± 1.12	8.47 ± 1.33[a]
PTH ng/liter	10.0–65.0	28.7 ± 3.8	20.0 ± 2.8[a]	25.1 ± 2.2	15.0 ± 1.9[a]
25OHD ng/ml	16.0–74.0	21.6 ± 4.8	18.1 ± 3.6	19.2 ± 2.7	17.5 ± 2.3
Total calcium mmol/liter	2.25–2.60	2.42 ± 0.03	2.45 ± 0.05	2.37 ± 0.04	2.53 ± 0.06[b]

* In doses of 1 μg/day and 2 μg/day for a month in two groups of osteoporotic women, evaluated by Student's paired *t*-test (means ± SEM). Initial values of all parameters did not differ significantly in both investigated groups (evaluated by Student's unpaired *t*-test).
[a] $P < 0.01$; [b] $P < 0.05$ (versus values before treatment)

of $1,25(OH)_2D_3$ in patients with osteoporosis stimulates circulating IGF-I, as well as β_2 microglobulin, in parallel with serum osteocalcin. The effect was documented at the higher dose of the secosteroid. The results concerning IGF-I are contradictory to the data of Finkelman et al. [17], which indicated that implants of bone matrix prepared from vitamin D-deficient rats had no effect on IGF-I production, as well as implants from vitamin D-replete animals. Moreover, Antoniazzi et al. [18] reported that serum IGF-I did not change in children with growth hormone deficiency after a 4-day treatment with $1,25(OH)_2D_3$ (in doses of 1.5 μg/day), although serum osteocalcin rose significantly. The inconsistent data may therefore be explained by the different sensitivity of osteoblasts to the sterol, dependent on species, age, dose, and the route of administration of vitamin D, as well as on the duration of vitamin D deficiency or treatment with sterol. Moreover, growth hormone status may also be of some importance for permission of the secosteroid action.

The present study was performed in winter, which explains the low normal means of $25OHD_3$ levels. Additionally, in three patients from each investigated group this parameter was below the lower limit of the normal range. Although vitamin D deficiency may alter the responsiveness of IGF-I and β_2 microglobulin to $1,25(OH)_2D_3$, the patients with low $25OHD_3$ did not differ in this respect from other participants. Thus, the sensitivity of the investigated systems to $1,25(OH)_2D_3$ seems to be independent of initial vitamin D status in the current study.

Bone-derived growth factor, which is also produced by hemopoietic cells, has been isolated from rat calvariae and identified as β_2 microglobulin [14]. The mediating role of this minor polypeptide in the action of bone anabolics has not previously been sufficiently investigated. In the isolated study by Cantatore et al. [19], a significant increase in serum β_2 microglobulin was observed after anabolic steroid decadurabolin. The authors postulated that androgens influence bone turnover at least partly via stimulation of serum vitamin D metabolites and subsequent increasing production of β_2 microglobulin by mononuclear cells. Therefore, the current data support this hypothesis.

An important stimulating effect of PTH on IGF-I production by osteoblasts has also been shown [10]. The relative decline of PTH levels is therefore effectively counterbalanced by the higher dose of $1,25(OH)_2D_3$, which strengthens the obtained data.

Response of bone-derived growth factors to osteotropic hormones is conditioned by the existence of specific receptors in skeletal tissue. The saturable high-affinity receptors for $1,25(OH)_2D_3$ have been found in human osteoblast-like cells or osteoclasts [8] (for review see [20]). It is known that the administration of $1,25(OH)_2D_3$ up-regulates these receptors in different tissues [21, 22] and stimulates serum osteocalcin levels, which is in agreement with the dose-dependent effect of the sterol shown by this study.

To conclude, it has been observed that $1,25(OH)_2D_3$ effectively stimulates the production of IGF-I and β_2 microglobulin, in parallel with osteocalcin, in a dose-dependent manner in patients with osteoporosis, a result not previously reported. The authors' data appears to support the hypothesis that IGF-I and β_2 microglobulin may partially mediate the bone remodeling effect of $1,25(OH)_2D_3$ in human porotic bones. As this effect occurs only with the higher therapeutic dose, a similar response could be attained by a long-term treatment with a lower dose; this remains to be confirmed.

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References

1. Canalis E, McCarthy T, Centrella M (1988) Isolation and characterization of insulin-like growth factor I (somatomedin-C) from cultures of fetal rat calvariae. Endocrinology 122:22–27
2. McCarthy TL, Centrella M, Canalis E (1989) Parathyroid hormone enhances the transcript and polypeptide levels of insulin-like growth factor I in osteoblast-enriched cultures from fetal rat bone. Endocrinology 124:1247–1253
3. Wong GL, Kotliar D, Schlaeger D, Brandes SJ (1990) IGF-I production by mouse osteoblasts. J Bone Miner Res 5:133–140
4. Okazaki R, Conover CA, Harris SA, Slelsberg TC, Riggs BL (1995) Normal human osteoblast-like cells consistently express genes for insulin-like growth factors I and II but transformed human osteoblast cell lines do not. J Bone Miner Res 10:788–795
5. Schmid Ch, Schläpfer I, Keller A, Waldvogel M, Froesch ER, Zapf J (1995) Effects of insulin-like growth factor (IGF) binding proteins (BPs)-3 and -6 on DNA synthesis of rat osteoblasts: further evidence for a role of auto-paracrine IGF I but not IGF II in stimulating osteoblast growth. Biochem Biophys Res Commun 212:242–248
6. Johansson AG, Lindh E, Ljunghall S (1992) Insulin-like growth factor I stimulates bone turnover in osteoporosis. Lancet 339:1619
7. Slootweg MC, Hoogerbrugge CM, de Poorter TL, Duursma

SA, van Buul-Offers SC (1990) The presence of classical insulin-like growth factor (IGF) type-I and -II receptors on mouse osteoblasts: autocrine/paracrine growth effect of IGFs? J Endocrinol 125:271–277
8. Liesegang P, Romalo G, Sudmann M, Wolf L, Schweikert H-U (1994) Human osteoblast-like cells contain specific, saturable, high-affinity glucocorticoid, androgen, estrogen, and 1α,25-dihydroxycholecalciferol receptors. J Androl 15:194–199
9. Beneton MN, Yates AJ, Rogers S, McCloskey EV, Kanis JA (1991) Stanozolol stimulates remodeling of trabecular bone and net formation of bone at the endocortical surface. Clin Sci Colch 81:543–549
10. Martin TJ, Allan EH, Fukumoto S, Moseley JM (1995) Some aspects of the anabolic action of parathyroid hormone. Miner Electrolyte Metab 21:87–90
11. Ernst M, Rodan GA (1991) Estradiol regulation of insulin-like growth factor-I expression in osteoblastic cells: evidence for transcriptional control. Mol Endocrinol 5:1081–1089
12. McCarthy TL, Centrella M, Canalis E (1992) Constitutive synthesis of insulin-like growth factor-II by primary osteoblast-enriched cultures from fetal rat calvariae. Endocrinology 130:1303–1308
13. Watson P, Lazowski D, Han V, Fraher L, Steer B, Hodsman A (1995) Parathyroid hormone restores bone mass and enhances osteoblast insulin-like growth factor I gene expression in ovariectomized rats. Bone 16:357–365
14. Canalis E, McCarthy T, Centrella M (1987) A bone-derived growth factor isolated from rat calvariae is $beta_2$ microglobulin. Endocrinology 121:1198–1200
15. Khoury RS, Weber J, Farach-Carson MC (1995) Vitamin D metabolites modulate osteoblast activity by Ca^{+2} influx-independent genomic and Ca^{+2} influx-dependent nongenomic pathways. J Nutr 125:1699S–1703S
16. Chen TL, Mallory JB, Hintz RL (1991) Dexamethasone and $1,25(OH)_2$vitamin D_3 modulate the synthesis of insulin-like growth factor-I in osteoblast-like cells. Calcif Tissue Int 48: 278–282
17. Finkelman RD, Linkhart TA, Mohan S, Lau KH, Baylink DJ, Bell NH (1991) Vitamin D deficiency causes a selective reduction in deposition of transforming growth factor beta in rat bone: possible mechanism for impaired osteoinduction. Proc Natl Acad Sci USA 88:3657–3660
18. Antoniazzi F, Radetti G, Zamboni G, Gambaro G, Adami S, Tato L (1993) Effects of 1,25-dihydroxyvitamin D_3 and growth hormone therapy on serum osteocalcin levels in children with growth hormone deficiency. Bone Miner 21:151–156
19. Cantatore FP, Loperfido MC, Mancini L, Carrozzo M (1992) Effect of calcitonin or the anabolic steroid decadurabolin on serum β_2 microglobulin in osteoporotic postmenopausal women. J Rheumatol 19:1753–1755
20. Hannah SS, Norman AW (1994) $1\alpha,25(OH)_2$ Vitamin D_3-regulated expression of the eukaryotic genome. Nutr Rev 52: 376–382
21. Lee S, Clark SA, Gill RK, Christakos S (1994) 1,25-dihydroxyvitamin D_3 and pancreatic β-cell function: vitamin D receptors, gene expression, and insulin secretion. Endocrinology 134:1602–1610
22. Davoodi F, Brenner RV, Evans SRT, Schumaker LM, Shabahang M, Nauta RJ, Bures RR (1995) Modulation of vitamin D receptor and estrogen receptor by $1,25(OH)_2$-vitamin D_3 in T-47D human breast cancer cells. J Steroid Biochem Molec Biol 54:147–153