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Eldecalcitol is less effective in suppressing parathyroid hormone compared to calcitriol *in vivo*☆

Suguru Harada, Satoshi Takeda, Akiko Uno, Fumiaki Takahashi, Hitoshi Saito*

Chugai Pharmaceutical Co., Ltd., 1-135 Komakado, Gotemba, Shizuoka 412-8513, Japan

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ABSTRACT

Eldecalcitol is a vitamin D_3 analog under clinical development for the treatment of osteoporosis. Previous studies have shown that the binding activities of eldecalcitol to the serum vitamin D binding protein (DBP) and the vitamin D receptor (VDR) are 421.9% and 44.6% of those of calcitriol, respectively, and also, suppressed parathyroid hormone (PTH) production by only 3.5% of calcitriol *in vitro* using bovine parathyroid cell primary culture. Here, we compared *in vivo* activities of eldecalcitol on serum calcium, BMD and PTH with those of calcitriol. Six-week old male rats were given either vehicle (medium chain triglyceride; $n=6$), eldecalcitol (0.025, 0.05, 0.1, 0.25, 0.5 μg/kg; $n=6$) or calcitriol (0.25, 0.5, 1.0, 2.5, 5 μg/kg; $n=6$) daily for 14 days by oral gavages. Eldecalcitol was approximately five-times more potent than calcitriol in increasing serum calcium. Eldecalcitol significantly increased lumbar spine BMD, however, calcitriol had no effect on BMD at any given doses. On the contrary, eldecalcitol did not affect PTH mRNA synthesis at the normocalcemic doses, despite the BMD was higher than normal. These observations indicate that, as previous *in vitro* study suggested, eldecalcitol is less effective in suppressing PTH compared to calcitriol.

1. Introduction

A previous clinical trial revealed that treatment of osteoporotic patients supplemented with 200 or 400 IU per day vitamin D_3 with 0.75 μg/day eldecalcitol for twelve months increased lumbar and hip bone mineral density (BMD) by 3.4% and 1.5%, respectively, compared with placebo group. The effect of eldecalcitol on bone was observed without sustained hypercalcemia or hypercalciuria [1]. The effects on BMD, bone turnover markers were stronger than any previous results using alfacalcidol or calcitriol [2,3].

In vivo animal studies using ovariectomized (OVX) rats indicated that eldecalcitol increased BMD greater than alfacalcidol by strongly suppressing bone resorption. Eldecalcitol decreased urinary excretion of deoxypyridinoline, and also decreased bone resorption parameters (eroded surface, osteoclast surface and osteoclast number) of the bone histomorphometrical analysis [4]. Meanwhile, some studies with various rat models indicated that eldecalcitol maintained bone formation [4–7]. Serum osteocalcin as well as bone formation parameters (bone formation rate and mineral apposition rate) in the bone histomorphometry stayed within the normal range during the eldecalcitol treatment. The binding activity of eldecalcitol to the serum vitamin D binding protein (DBP) is slightly higher than that of calcitriol, but the binding to the vitamin D receptor (VDR) is slightly lower than that of calcitriol *in vitro* [8]. These characteristics may explain its longer half-life in the circulation [8] and poorer distribution to the target organs than calcitriol (unpublished data). Also, eldecalcitol hardly suppresses parathyroid hormone (PTH) production compared with calcitriol *in vitro* using bovine parathyroid cell primary culture [9]. Here, we compared *in vivo* activities of eldecalcitol with calcitriol on serum calcium, BMD and PTH.

2. Material and method

2.1. Animals

Sixty-six of six week old male Sprague-Dawley rats were maintained under specific pathogen free condition with 12-h light and dark cycle, fed *ad libitum* with normal rat chow (CE-2, CLEA Japan, Japan) and tap water. After one-week acclimation, rats were randomly divided to eleven groups ($n=6$ each), then administered either vehicle (medium chain triglyceride), eldecalcitol (0.025, 0.05, 0.1, 0.25, 0.5 μg/kg) or calcitriol (0.25, 0.5, 1.0, 2.5, 5 μg/kg) daily for 14 days by oral gavages. Blood, bones and parathyroid samples were taken at six hours after the last dosing. The studies were reviewed and approved by the Institutional Animal Care and Use of Committee in Chugai Pharmaceutical Co., Ltd.

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* Corresponding author at: Lifecycle Management Department 2, Chugai Pharmaceutical Co., Ltd., 2-1-1 Nihonbashi-Muromachi, Chuo-ku, Tokyo 103-8324, Japan. Tel.: +81 3 3516 5299; fax: +81 3 3281 0191.
E-mail address: saitohts@chugai-pharm.co.jp (H. Saito).

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2.2. Bone mineral density

BMD was measured by dual-energy X-ray absorptiometry (DCS-600EX, Aloka, Japan) at the second to fifth vertebrae.

2.3. Serum calcium

Serum calcium was measured with an automatic analyzer (TBA-120FR, Toshiba Medical Systems, Japan).

2.4. Plasma PTH

Plasma PTH was measured by using rat PTH ELISA (Rat Intact PTH ELISA Kit, Immutopics, USA).

2.5. Quantitative RT-PCR

Excised parathyroid/thyroid glands were frozen in the liquid nitrogen. Frozen tissues were soaked into the TRIzol (Invitrogen, USA) and immediately crushed with homogenizer (Physcotron, Microtec Nition, Japan) on ice. Total RNA was extracted with RNeasy Mini Kit (QIAGEN, Germany). Quantitative RT-PCR was carried out by ABI-PRISM system (PRISM7700, ABI, USA)

2.6. Statistical analysis

Data represented the mean ± standard error (SE). Statistical analysis was performed using the SAS System for Windows (SAS Institute, NC, USA). Statistical significance was determined by Dunnett's *t*-test.

3. Results

3.1. Serum calcium and BMD

Eldecalcitol dose-dependently increased serum calcium, approximately five times more potent than calcitriol (Fig. 1). Serum calcium was significantly elevated at the higher doses of eldecalcitol (0.25 and 0.5 μg/kg) and calcitriol (1.0, 2.5 and 5 μg/kg), but unchanged at lower doses of eldecalcitol (0.025, 0.05 and 0.1 μg/kg) and of calcitriol (0.25 and 0.5 μg/kg). Lumbar spine BMD significantly increased at the 0.05 and 0.1 μg/kg in the eldecalcitol treated groups (Fig. 2), however, calcitriol did not increase BMD at any given doses in

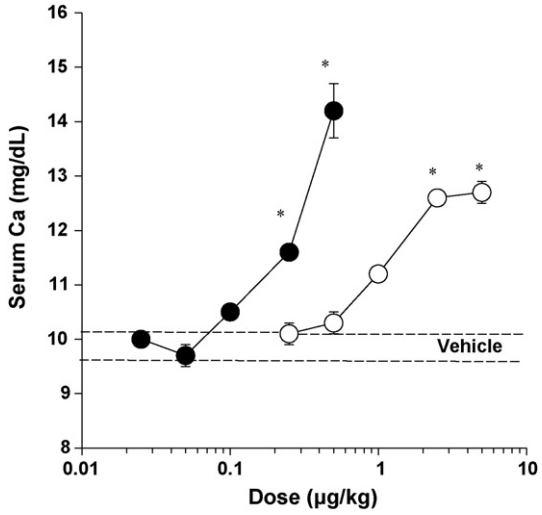

Fig. 1. Serum calcium in either eldecalcitol or calcitriol treated rats. Data represent mean ± SE ($n = 6$). The dotted lines indicate a normal range (vehicle control). *$P < 0.05$ vs. vehicle control by Dunnet's multiple test.

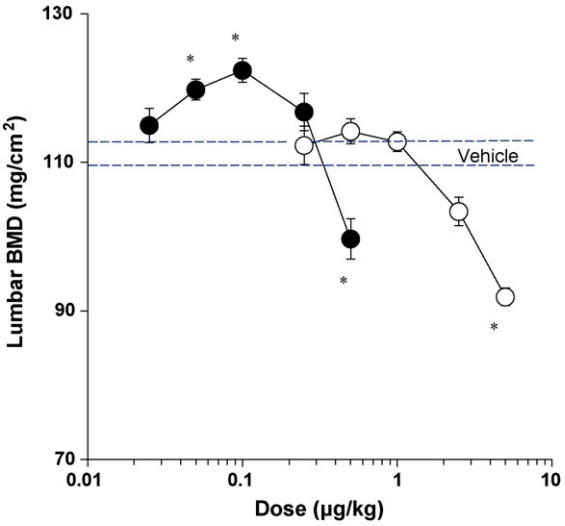

Fig. 2. Lumbar spine BMD (L_2–L_5) in either eldecalcitol or calcitriol treated rats. Data represent mean ± SE ($n = 6$). The dotted lines indicate a normal range (vehicle control). *$P < 0.05$ vs. vehicle control by Dunnet's multiple test.

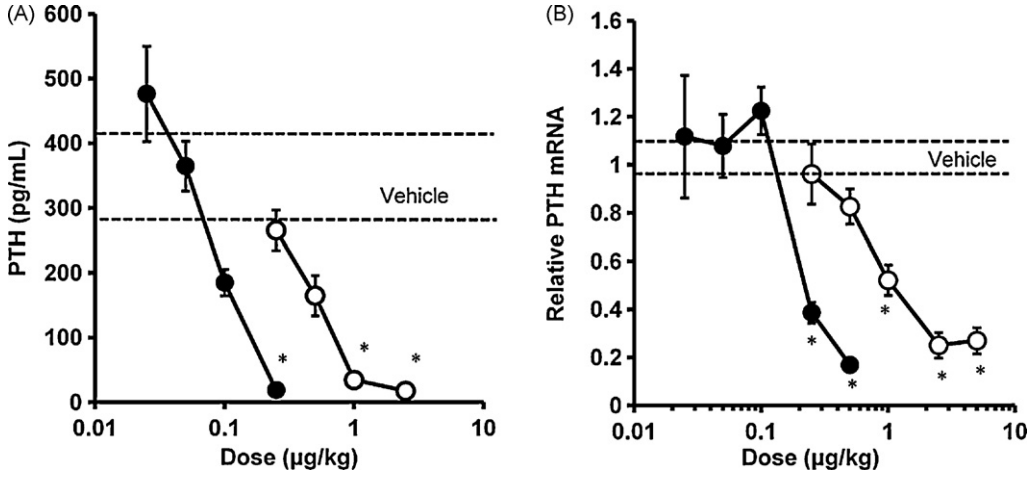

Fig. 3. Plasma concentration of PTH (A) and relative amount of PTH mRNA (B) in parathyroid gland in either eldecalcitol or calcitriol treated rats. Data represent mean ± SE ($n = 6$). The dotted lines indicate a normal range (vehicle control). *$P < 0.05$ vs. vehicle control by Dunnet's multiple test.

this study. The BMD became below the normal range at the higher doses, 2.5 and 5 μg/kg of calcitriol as well as 0.5 μg/kg of eldecalcitol.

3.2. Effects on plasma PTH and PTH mRNA biosynthesis in PTG

Eldecalcitol and calcitriol dose dependently suppressed plasma PTH. Eldecalcitol was approximately five-times more potent than calcitriol (Fig. 3A). However, normocalcemic dose (0.025, 0.05, and 0.1 μg/kg) of eldecalcitol did not affect PTH mRNA transcription in the parathyroid gland (Fig. 3B).

4. Discussion

Eldecalcitol increased BMD and reduce bone turnover markers in normal, ovariectomized and steroid-treated rats [4,6,7], also, in patients with osteoporosis [1]. Alfacalcidol, calcitriol and eldecalcitol suppressed bone resorption in OVX rats *in vivo* by reducing the activity and number of osteoclasts [4,10,11]. Calcitriol directly inhibits maturation/differentiation of the osteoclast by diminishing c-Fos protein in the osteoclast precursors [12]. Here, we again showed that eldecalcitol significantly increased BMD without causing hypercalcemia in normal rats. On the other hand, calcitriol did not increase BMD at any dose in this study.

Both calcitriol and eldecalcitol increased serum calcium and reduced plasma PTH. Calcitriol suppressed PTH mRNA synthesis and reduced plasma PTH from the lowest dose (0.25 μg/kg) to the highest dose (5 μg/kg), dose-dependently. However, eldecalcitol did not affect PTH mRNA synthesis at the normocalcemic dose (0.025, 0.05 and 0.1 μg/kg). Plasma PTH was above the normal level at 0.025 and 0.05 μg/kg, and then dose-dependently reduced at higher doses (0.25 and 0.5 μg/kg) of eldecalcitol. These observations indicate that eldecalcitol does not suppress PTH gene transcription at the normocalcemic doses which eldecalcitol increases BMD in rat *in vivo*. Therefore, eldecalcitol is less active in suppressing PTH gene transcription than calcitriol *in vivo*.

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