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Suppression of PTH by the Vitamin D Analog Eldecalcitol Is Modulated by its High Affinity for the Serum Vitamin D-Binding Protein and Resistance to Metabolism

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ABSTRACT

Eldecalcitol [1α,25-dihydroxy-2β-(3-hydroxypropyloxy)vitamin D_3], a vitamin D analog with enhanced efficacy for treatment of osteoporosis, has been found to be less potent than 1,25-dihydroxyvitamin D_3 (calcitriol) in suppressing PTH in vivo. To define the mechanism for the latter observation, we compared the effects of eldecalcitol and calcitriol on PTH secretion by bovine parathyroid cells. While the two compounds showed similar potency when the cells were cultured in medium containing 15% newborn calf serum, eldecalcitol was 100 times more potent than calcitriol in the absence of serum. Eldecalcitol has a higher affinity for the serum vitamin D-binding protein (DBP), and therefore binding to DBP, and possibly other serum components, appears to limit the uptake and activity of eldecalcitol in parathyroid cells, providing an explanation for the lower PTH suppressing activity in vivo (100% serum). However, the 100-fold higher activity of eldecalcitol in the absence of serum was unexpected since the VDR affinity for eldecalcitol is eightfold lower than for calcitriol. The enhanced activity was not due to preferential uptake, but to a resistance to metabolism. While 1 nM [^{3}H]calcitriol was completely degraded within 24 h, [^{3}H]eldecalcitol was not metabolized, despite the induction of the vitamin D catabolic enzyme, 24-hydroxylase (CYP24A). The resistance to metabolism is the likely explanation for the higher potency of eldecalcitol in suppressing PTH in cell culture lacking serum. Thus, the unique properties of eldecalcitol in vivo can be attributed, at least in part, to its high-DBP affinity which increases the half-life, but limits the uptake of eldecalcitol, and to its reduced metabolism, which prolongs the activity of this analog in target tissues. J. Cell. Biochem. 112: 1348–1352, 2011.

KEY WORDS: PARATHYROID HORMONE; VITAMIN D ANALOG; VITAMIN D-BINDING PROTEIN; VITAMIN D-24-HYDROXYLASE; CATABOLISM

Osteoporosis is a therapeutic target for vitamin D compounds. The active hormonal form of vitamin D, 1,25-dihydroxyvitamin D_3 (calcitriol), and its synthetic analog, 1α-hydroxyvitamin D_3 (alfacalcidol), have been used with success to slow mineral loss, but a major goal of vitamin D research is to find analogs that produce an increase in bone mineral. One such analog is 1,25-dihydroxy-2β-(3-hydroxypropyloxy)-vitamin D_3 (eldecalcitol). This analog was shown to stimulate bone mineralization in ovariectomized rats [Nishii et al., 1993; Tsurukami et al., 1994; Ono et al., 1998; Uchiyama et al., 2002], in corticosteroid-treated rats [Tanaka et al., 1996], and in bone marrow-ablated rats [Okuda et al., 2007]. Clinical trials demonstrated that eldecalcitol increased bone mass in osteoporotic patients and was superior to alfacalcidol [Kubodera et al., 2003; Matsumoto et al., 2005; Matsumoto and Kubodera, 2007; Kubodera, 2009]. The explanation for the greater effectiveness eldecalcitol is not known. Vitamin D compounds can affect bone mineral content by direct action on the bone and indirectly by altering the levels of calcium and phosphate and the calciotropic hormone PTH. Chronically elevated PTH levels stimulate bone turnover with a net loss of mineral.

The properties of eldecalcitol that are responsible for the favorable effects on bone are not clear. Its affinity for the vitamin D receptor (VDR) is 8 times lower than that of calcitriol [Okano et al., 1989]. This difference likely does not explain the enhanced bone mineralization effects, but induction by eldecalcitol of a VDR conformation with distinct transcriptional activities cannot be excluded. On the other hand, eldecalcitol has higher affinity for the serum vitamin D-binding protein (DBP) [Okano et al., 1989; Hatakeyama et al., 2007], which may increase its circulating half-life while limiting its rate of uptake into target tissues. Furthermore, other vitamin D analogs with substitutions at the 2-position are poor substrates for the vitamin D-24-hydroxylase (CYP24A), the major catabolic enzyme for vitamin D compounds [Flanagan et al., 2009; Saito et al., 2009]. Resistance to metabolism could enhance and

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prolong the cellular actions of eldecalcitol. The altered pharmacokinetics may play a role in the beneficial effects of eldecalcitol on bone, but their specific impact on parathyroid cell function has not been investigated. In the present study, we investigate the potential roles of DBP binding and resistance to metabolism on the activities of eldecalcitol in cultured bovine parathyroid cells.

METHODS

PREPARATION OF DISPERSED BOVINE PARATHYROID CELLS

Dispersed bovine parathyroid cells were prepared as previously described [Brown et al., 1992]. Briefly, bovine parathyroid glands (obtained from Pel-Freez Biologicals) were trimmed of extraneous fatty tissue, sliced to 0.5 mm thickness with a tissue slicer (Stadie Riggs; Thomas Scientific, Swedesboro, NJ) and placed in a mixture of DME:Ham's F-12 medium (50:50) containing 0.5 mM calcium and collagenase (3,000 U/ml of collagenase XI-S; Sigma, St. Louis). The suspension (10 ml media per gram of tissue) was agitated in a shaking water bath at 37°C for 90 min. Periodic passage of the mixture through the tip of a 10 ml pipette assisted in the disaggregation. The digested tissue was washed three times with serum-free culture medium containing DME:Ham's F-12 (50:50), 1 mM $CaCl_2$, 15 mM Hepes, 100 IU/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml insulin, 5 μg/ml holo-transferrin, 2 mM glutamine, 1% nonessential amino acids, and 0.1% bovine serum albumin (fraction V). Dispersed cells were plated in 24-well culture plates at 160,000 cells/well in the above medium containing 4% newborn calf serum. After 24 h, the medium was replaced with either serum-free medium or medium containing 15% newborn calf serum.

REGULATION OF PTH RELEASE BY bPTC

Primary cultures of bPTC were grown to confluency in serum-free medium as previously described and then treated for 72 h with 0.1, 1.0, 10, 100 nM eldecalcitol or calcitriol, with daily changes of medium. The cells were then changed into fresh medium and the amount of PTH secreted during a 2-h incubation was determined using an intact bovine PTH ELISA kit (Immutopics, San Capistrano, CA).

UPTAKE BY PARATHYROID CELLS

The relative rates and extent of uptake of eldecalcitol and calcitriol by the cultured bovine parathyroid cells were determined using [^{3}H]-calcitriol (Perkin-Elmer) and [^{3}H]-eldecalcitol (Chugai Pharmaceuticals). The cells were grown to near confluence and the medium was replaced with serum-free medium containing 0.1% BSA and the tritiated compounds (1 nM, 100,000 DPM). After 0, 10, 30, 60, and 120 min ($n = 3$), the medium was removed, and the cells were placed on ice and quickly washed twice with ice-cold PBS. The cells were lysed in 1 ml of 0.1 M NaOH, and the lysate was neutralized and analyzed for tritium using an ICN Micromedic Taurus scintillation counter.

METABOLISM BY PARATHYROID CELLS

The relative rates of metabolism of eldecalcitol and calcitriol by bovine parathyroid cells were determined using the tritiated compounds. The cells were incubated as above in 12-well plates with 1 ml of medium containing tritiated eldecalcitol or calcitriol (1 nM, 0.1 μCi) for 0, 4, 8, and 24 h. The cells plus medium were extracted by a modification of the method of Bligh and Dyer [1959]. The incubations were stopped by addition of 1 ml of mildly alkaline methanol (1 drop of 14.8 N ammonium hydroxide/100 ml methanol) containing 500 pmol of radioinert eldecalcitol or calcitriol to gauge recovery. The cells were scraped and transferred to a screw-cap tube. The well was washed with 1.5 ml methanol which was transferred to the tube along with 1 ml water and 2.5 ml methylene chloride. After shaking, the organic phase was removed and the aqueous phase was re-extracted with 2.5 ml methylene chloride. The combined organic phases were dried under nitrogen and dissolved in 250 μl of hexane:isopropanol:methanol (92:7:1) for HPLC analysis. The aqueous phase was centrifuged, and 1 ml was directly analyzed for tritium to estimate the water-soluble side-chain cleavage end-products. HPLC analysis was performed with a Beckman LC388 using a 0.46 cm × 25 cm Zorbax-Sil column (Phenomenex) and a mobile phase of hexane:isopropanol:methanol (92:7:1). Non-metabolized substrate, detected by ultraviolet absorption (265 nm) of the internal standard, was collected and analyzed for tritium.

RT-PCR OF VITAMIN D 24-HYDROXYLASE (CYP24A1) IN bPTC

Total RNA was isolated using RNAzol Bee (Cinna/Biotecx). Reverse transcription of the RNA was carried out using oligo-dT primer and reverse transcriptase (SMART MMLV; Clontech) as directed. qPCR was performed using SYBR Green (Sigma) in a Perkin Elmer Applied Biosystems Gene Amp 5700 Sequence Detection System. The primers for qPCR for bovine CYP24A1 were 5′-TGG TCG CCG GTT AGC TGA ACT C-3′ and 5′-CCA AAT GCA GCG TCT CCA CAG-3′ (106 bp). Parallel amplifications were performed with primers for the housekeeping gene glyceraldehydes-3-phosphate dehydrogenase 5′-TCT GAC GCC TGC TTC ACC AC-3′ and 5′-GCT CAA CGG GAA GCT CAC TG-3′ (132 bp). The data are expressed as the ratio of CYP24A1 to GAPDH mRNA ($\Delta\Delta C_t$ method).

STATISTICS

The activities, uptake, and metabolism of eldecalcitol and calcitriol were analyzed by ANOVA, with $P < 0.05$ as significant.

RESULTS

The relative potencies of eldecalcitol and calcitriol to suppress PTH release from bovine parathyroid cells grown in the presence of 15% newborn calf serum is shown in Figure 1. Under these conditions, the two compounds were roughly equivalent in their efficacies to reduce PTH secretion. However, eldecalcitol has a higher affinity for the serum DBP, and its uptake by parathyroid may be hindered by serum. To test this hypothesis, the potencies of eldecalcitol and calcitriol were compared in bovine parathyroid cells cultured in the absence of serum. As shown in Figure 2, under serum-free conditions, eldecalcitol was approximately 100 times more potent than calcitriol, indicating that serum, presumably DBP, hinders the activity of eldecalcitol to suppress PTH synthesis and secretion.

Relative rates of uptake of eldecalcitol and calcitriol by bovine parathyroid cells were determined using tritiated compounds. Initial

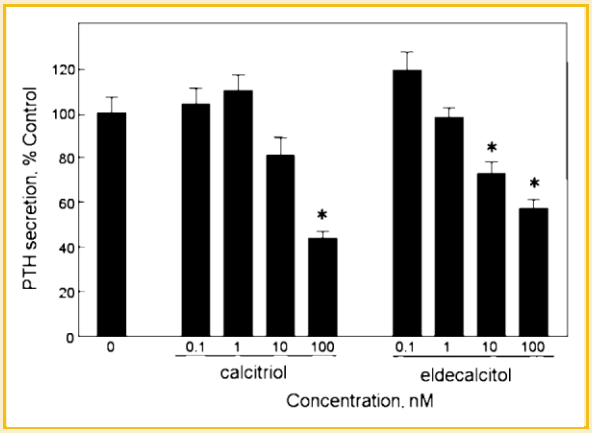

Fig. 1. Suppression of PTH by eldecalcitol and calcitriol in bovine parathyroid cells cultured in the presence of 15% serum. Bovine parathyroid cell monolayers were cultured in medium containing 15% newborn calf serum and treated for 3 days with the specified concentrations of eldecalcitol or calcitriol. Steady-state PTH release was measured as described in Methods Section. Data are presented as mean ± SEM (n = 6). $^{*}P < 0.05$ versus control.

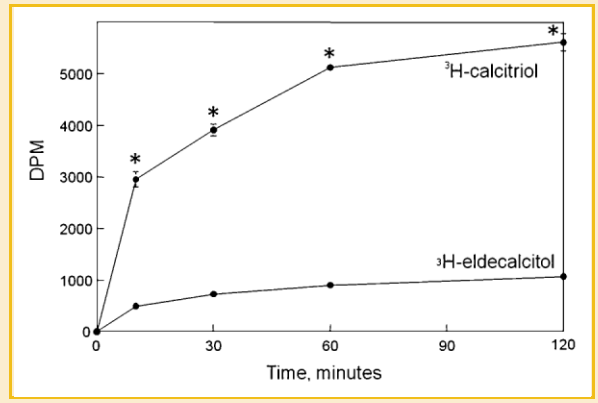

Fig. 3. Uptake of eldecalcitol and calcitriol by bovine parathyroid cells. Bovine parathyroid cell monolayers were cultured in serum-free medium and then incubated with 1 nM [^{3}H]eldecalcitol or [^{3}H]calcitriol for the specified time. Cell-associated tritium was determined as described in Methods Section. Data are presented as mean ± SEM (n = 6). $^{*}P < 0.05$ eldecalcitol versus calcitriol.

studies were performed under serum-free conditions. As shown in Figure 3, uptake of eldecalcitol was much lower than that of calcitriol, even in the absence of serum (i.e., DBP). Thus, the 100-fold higher activity of eldecalcitol under serum-free conditions (Fig. 2) cannot be attributed to enhanced uptake.

Since the affinity of eldecalcitol for the VDR is approximately 8 times lower than that of calcitriol, another explanation was sought. We compared the rates of metabolism of eldecalcitol and calcitriol using tritiated compounds. Bovine parathyroid cells were maintained in serum-free medium and incubated with 1 nM tritiated eldecalcitol or calcitriol for up to 24 h, and the amount of parent compound remaining was assessed by HPLC. As shown in Figure 4, calcitriol was completely degraded by the end of the incubation, while eldecalcitol was not significantly metabolized. These finding were confirmed by analysis of the water-soluble end-products, which showed accumulation of radioactivity in the aqueous phases with time from calcitriol incubations, but no accumulation in the eldecalcitol samples. Degradation of vitamin D compounds is accomplished primarily by the 24-hydroxylase (CYP24A1), which is induced transcriptionally by activation of the VDR. Figure 5 shows that 1 nM eldecalcitol was much more potent than calcitriol in inducing CYP24A1 mRNA in the absence of serum, but the effects of

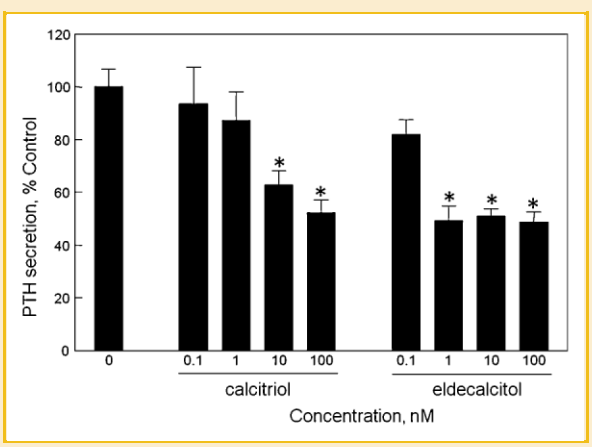

Fig. 2. Suppression of PTH by eldecalcitol and calcitriol in bovine parathyroid cells cultured in the absence of serum. Bovine parathyroid cell monolayers were cultured in serum-free medium and treated for 3 days with the specified concentrations of eldecalcitol or calcitriol. Steady-state PTH release was measured as described in Methods Section. Data are presented as mean ± SEM SEM (n = 12). $^{*}P < 0.05$ versus control.

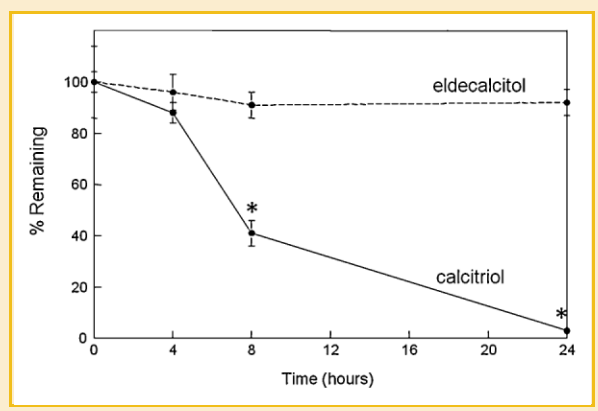

Fig. 4. Metabolism of eldecalcitol and calcitriol by bovine parathyroid cells. Bovine parathyroid cell monolayers were cultured in serum-free medium and then incubated with 1 nM [^{3}H]eldecalcitol or [^{3}H]calcitriol for the specified time. Cells plus medium were extracted and the organic phase was dried and resolved by normal phase HPLC as described in Methods Section to determine the percent of the parent compound remaining. Data are presented as mean ± SEM (n = 6). $^{*}P < 0.05$ eldecalcitol versus calcitriol.

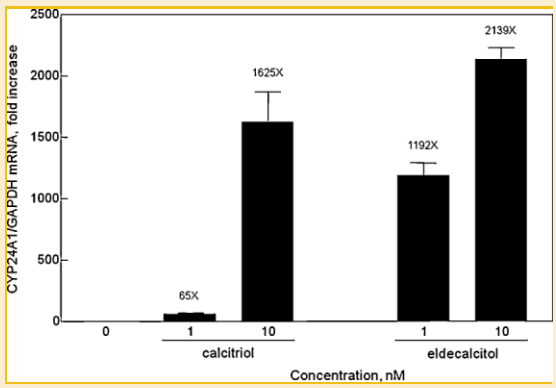

Fig. 5. Induction of parathyroid cells 24-hydroxylase (CYP24A1) by eldecalcitol and calcitriol. Bovine parathyroid cell monolayers were cultured in serum-free medium and then incubated with vehicle (ethanol) or with 1 nM eldecalcitol or calcitriol for 6 h. RNA was extracted and reverse transcribed, and the CYP24A1 and GAPDH mRNA were quantified by qPCR as described in Methods Section. Data are presented as mean ± SEM (n = 4).

both compounds were nearly maximal at 100 nM. This finding is similar to the effects on PTH secretion (Fig. 2), indicating that eldecalcitol acts as a superagonist in parathyroid cells under these conditions. The resistance to metabolism even in the presence of highly induced 24-hydroxylase suggests that the hydroxypropyloxy group at the 2-position interferes with binding and/or oxidation by CYP24A1. This stability of eldecalcitol in parathyroid culture likely provides the explanation for the higher than expected PTH-suppressing activity, based on its lower VDR affinity.

DISCUSSION

Eldecalcitol shows tremendous promise for treatment of osteoporosis. Studies in both ovariectomized rats and patients with osteoporosis demonstrated that this vitamin D analog can increase bone mass by increasing bone formation and reducing bone resorption, whereas similar studies and trials showed that calcitriol and alfacalcidol only slow or prevent further loss of bone mass [Nishii et al., 1993; Tsurukami et al., 1994; Tanaka et al., 1996; Ono et al., 1998; Uchiyama et al., 2002; Kubodera et al., 2003; Matsumoto et al., 2005; Matsumoto and Kubodera, 2007; Okuda et al., 2007; Kubodera, 2009]. The reason for the greater beneficial effects of eldecalcitol on bone, compared to calcitriol and alfacalcidol, is not clear, but may be attributable to differences in pharmacokinetics. The present study examined the relative actions of calcitriol and eldecalcitol in an important vitamin D target, parathyroid cells.

Eldecalcitol has been shown to bind more avidly to DBP than calcitriol [Okano et al., 1989; Hatakeyama et al., 2007], which would lead to a longer half-life in vivo, but reduce its uptake by target cells expressing the VDR. In the present study, the presence of serum reduced the PTH-suppressing activity of eldecalcitol relative to calcitriol. The two compounds were equally active in 15% serum, but eldecalcitol was about 100 times more active in the absence of serum. The effect of DBP in serum-containing medium on the activities of vitamin D compounds is well-established [Bouillon et al., 1991; Dusso et al., 1991; Dilworth et al., 1994; Zella et al., 2008]. The reported lower activity of eldecalcitol compared to calcitriol in suppressing PTH in vivo [Harada et al., 2010] is consistent with the greater inhibitory effect of DBP on uptake of eldecalcitiol by parathyroid cells.

The 100-fold higher potency of eldecalcitol to suppress PTH in the absence of serum was surprising given its eightfold lower affinity for the VDR [Okano et al., 1989]. However, metabolism studies revealed that eldecalcitol was resistant to degradation in the parathyroid cultures. We have previously demonstrated that bovine parathyroid cells rapidly degrade calcitriol, and this quickly reduces the levels of calcitriol–VDR complexes [Brown et al., 1992]. Subsequent studies confirmed that, as in other target cells, calcitriol induced the vitamin D-24-hydroxylase (CYP24A1) which catalyzes consecutive oxidation reactions of vitamin D side chain, leading to cleavage and inactivation. Eldecalcitol induced CYP24A1, but was not degraded by the enzyme. This is very likely the explanation for its higher-than-expected activity. This has been shown to be the case for several other vitamin D analogs with modifications of the 2-position. Flanagan et al. [2009] reported that 19-nor-2α-hydroxypropyl-1,25$(OH)_2D_3$ was much more potent than calcitriol in inhibiting prostate cell growth in vitro, which they attributed to the 50-fold slower metabolism of this analog by the 24-hydroxylase. Saito et al. [2009] found that 2α-propoxy-1,25$(OH)_2D_3$ was metabolized more slowly than 1,25$(OH)_2D_3$. Several groups have demonstrated that 2α-substituted vitamin D analogs are more active than predicted by their VDR affinity, even when tested in vitro [Glebocka et al., 2006; Shimizu et al., 2006; Glebocka et al., 2007; Kobayashi et al., 2007; Flanagan et al., 2009; Saito et al., 2009], probably because the stability of these compounds. Resistance to metabolism also has been implicated in the greater potencies or more prolonged effects of many other vitamin D analogs modified in other positions [Dilworth et al., 1994; Zhao et al., 1996; Hansen and Maenpaa, 1997; Kissmeyer et al., 1997; Shankar et al., 1997; Lechner et al., 2007].

The present findings illustrate the influences of higher DBP affinity and resistance to target cell metabolism on the activity of eldecalcitol using bovine parathyroid cells. The higher DBP affinity likely alters the pharmacokinetics of eldecalcitol by prolonging its circulating half-life while limiting its cellular uptake. However, the limited uptake of eldecalcitol is compensated by its resistance to degradation, which will extend and enhance its activity. These unique pharmacokinetic properties may be responsible for the beneficial effects of eldecalcitol on bone metabolism that has led to its development as a therapy for osteoporosis. Further studies are necessary to dissect the individual effects of DBP binding and resistance to metabolism on the activities of eldecalcitol in bone and other calcium-regulating tissues.

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REFERENCES

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37(8):911–917.

Bouillon R, Allewaert K, Xiang DZ, Tan BK, van Baelen H. 1991. Vitamin D analogs with low affinity for the vitamin D binding protein: Enhanced in vitro and decreased in vivo activity. J Bone Miner Res 6(10):1051–1057.

Brown AJ, Berkoben M, Ritter CS, Slatopolsky E. 1992. Binding and metabolism of 1,25-dihydroxyvitamin D_3 in cultured bovine parathyroid cells. Endocrinology 130(1):276–281.

Dilworth FJ, Calverley MJ, Makin HL, Jones G. 1994. Increased biological activity of 20-epi-1,25-dihydroxyvitamin D3 is due to reduced catabolism and altered protein binding. Biochem Pharmacol 47(6):987–993.

Dusso AS, Negrea L, Gunawardhana S, Lopez-Hilker S, Finch J, Mori T, Nishii Y, Slatopolsky E, Brown AJ. 1991. On the mechanisms for the selective action of vitamin D analogs. Endocrinology 128(4):1687–1692.

Flanagan JN, Zheng S, Chiang KC, Kittaka A, Sakaki T, Nakabayashi S, Zhao X, Spanjaard RA, Persons KS, Mathieu JS, Holick MF, Chen TC. 2009. Evaluation of 19-nor-2alpha-(3-hydroxypropyl)-1alpha,25-dihydroxyvitamin D3 as a therapeutic agent for androgen-dependent prostate cancer. Anticancer Res 29(9):3547–3553.

Glebocka A, Sicinski RR, Plum LA, Clagett-Dame M, DeLuca H. 2006. New 2-alkylidene 1alpha,25-dihydroxy-19-norvitamin D3 analogues of high intestinal activity: Synthesis and biological evaluation of 2-(3′-alkoxypropylidene) and 2-(3′-hydroxypropylidene) derivatives. J Med Chem 49(10): 2909–2920.

Glebocka A, Sicinski RR, Plum LA, DeLuca HF. 2007. 2-(3′-Hydroxypropylidene)-1alpha-hydroxy-19-norvitamin D compounds with truncated side chains. J Steroid Biochem Mol Biol 103(3–5):310–315.

Hansen CM, Maenpaa PH. 1997. EB1089, a novel vitamin D analog with strong antiproliferative and differentiation-inducing effects on target cells. Biochem Pharmacol 54:1173–1179.

Harada S, Takeda S, Uno A, Takahashi F, Saito H. 2010. Eldecalcitol is less effective in suppressing parathyroid hormone compared to calcitriol in vivo. J Steroid Biochem Mol Biol 121(1–2):281–283.

Hatakeyama S, Nagashima S, Imai N, Takahashi K, Ishihara J, Sugita A, Nihei T, Saito H, Takahashi F, Kubodera N. 2007. Synthesis and biological evaluation of a 3-positon epimer of 1alpha,25-dihydroxy-2beta-(3-hydroxypropoxy)vitamin D3 (ED-71). J Steroid Biochem Mol Biol 103(3–5):222–226.

Kissmeyer AM, Binderup E, Binderup L, Mork Hansen C, Andersen NR, Makin HL, Schroeder NJ, Shankar VN, Jones G. 1997. Metabolism of the vitamin D analog EB 1089: Identification of in vivo and in vitro liver metabolites and their biological activities. Biochem Pharmacol 53(8):1087–1097.

Kobayashi E, Shimazaki M, Miyamoto Y, Masuno H, Yamamoto K, DeLuca HF, Yamada S, Shimizu M. 2007. Structure–activity relationships of 19-norvitamin D analogs having a fluoroethylidene group at the C-2 position. Bioorg Med Chem 15(3):1475–1482.

Kubodera N. 2009. D-hormone derivatives for the treatment of osteoporosis: From alfacalcidol to eldecalcitol. Mini Rev Med Chem 9(12):1416–1422.

Kubodera N, Tsuji N, Uchiyama Y, Endo K. 2003. A new active vitamin D analog, ED-71, causes increase in bone mass with preferential effects on bone in osteoporotic patients. J Cell Biochem 88(2):286–289.

Lechner D, Manhardt T, Bajna E, Posner GH, Cross HS. 2007. A 24-phenylsulfone analog of vitamin D inhibits 1alpha,25-dihydroxyvitamin D(3) degradation in vitamin D metabolism-competent cells. J Pharmacol Exp Ther 320(3):1119–1126.

Matsumoto T, Kubodera N. 2007. ED-71, a new active vitamin D3, increases bone mineral density regardless of serum 25(OH)D levels in osteoporotic subjects. J Steroid Biochem Mol Biol 103(3–5):584–586.

Matsumoto T, Miki T, Hagino H, Sugimoto T, Okamoto S, Hirota T, Tanigawara Y, Hayashi Y, Fukunaga M, Shiraki M, Nakamura T. 2005. A new active vitamin D, ED-71, increases bone mass in osteoporotic patients under vitamin D supplementation: A randomized, double-blind, placebo-controlled clinical trial. J Clin Endocrinol Metabol 90(9):5031–5036.

Nishii Y, Sato K, Kobayashi T. 1993. The development of vitamin D3 analogues for the treatment of osteoporosis. Osteoporos Int 3(Suppl 1): 190–193.

Okano T, Tsugawa N, Masuda S, Takeuchi A, Kobayashi T, Takita Y, Nishii Y. 1989. Regulatory activities of 2 beta-(3-hydroxypropoxy)-1 alpha, 25-dihydroxyvitamin D3, a novel synthetic vitamin D3 derivative, on calcium metabolism. Biochem Biophys Res Commun 163(3):1444–1449.

Okuda N, Takeda S, Shinomiya K, Muneta T, Itoh S, Noda M, Asou Y. 2007. ED-71, a novel vitamin D analog, promotes bone formation and angiogenesis and inhibits bone resorption after bone marrow ablation. Bone 40(2):281–292.

Ono Y, Kawase A, Watanabe H, Shiraishi A, Takeda S, Higuchi Y, Sato K, Yamauchi T, Mikami T, Kato M, Tsugawa N, Okano T, Kubodera N. 1998. Syntheses and preventive effects of analogues related to 1alpha,25-dihydroxy-2beta-(3-hydroxypropoxy)vitamin D3 (ED-71) on bone mineral loss in ovariectomized rats. Bioorg Med Chem 6(12):2517–2523.

Saito N, Suhara Y, Abe D, Kusudo T, Ohta M, Yasuda K, Sakaki T, Honzawa S, Fujishima T, Kittaka A. 2009. Synthesis of 2alpha-propoxy-1alpha,25-dihydroxyvitamin D3 and comparison of its metabolism by human CYP24A1 and rat CYP24A1. Bioorg Med Chem 17(13):4296–4301.

Shankar VN, Dilworth FJ, Makin HL, Schroeder NJ, Trafford DJ, Kissmeyer AM, Calverley MJ, Binderup E, Jones G. 1997. Metabolism of the vitamin D analog EB1089 by cultured human cells: Redirection of hydroxylation site to distal carbons of the side-chain [published erratum appears in Biochem Pharmacol 1997 Jun 15;53(12):1946]. Biochem Pharmacol 53(6):783–793.

Shimizu M, Miyamoto Y, Kobayashi E, Shimazaki M, Yamamoto K, Reischl W, Yamada S. 2006. Synthesis and biological activities of new 1alpha,25-dihydroxy-19-norvitamin D3 analogs with modifications in both the A-ring and the side chain. Bioorg Med Chem 14(12):4277–4294.

Tanaka Y, Nakamura T, Nishida S, Suzuki K, Takeda S, Sato K, Nishii Y. 1996. Effects of a synthetic vitamin D analog, ED-71, on bone dynamics and strength in cancellous and cortical bone in prednisolone-treated rats. J Bone Miner Res 11(3):325–336.

Tsurukami H, Nakamura T, Suzuki K, Sato K, Higuchi Y, Nishii Y. 1994. A novel synthetic vitamin D analogue, 2 beta-(3-hydroxypropoxy)1 alpha, 25-dihydroxyvitamin D3 (ED-71), increases bone mass by stimulating the bone formation in normal and ovariectomized rats. Calcif Tissue Int 54(2):142–149.

Uchiyama Y, Higuchi Y, Takeda S, Masaki T, Shira-Ishi A, Sato K, Kubodera N, Ikeda K, Ogata E. 2002. ED-71, a vitamin D analog, is a more potent inhibitor of bone resorption than alfacalcidol in an estrogen-deficient rat model of osteoporosis. Bone 30:582–588.

Zella LA, Shevde NK, Hollis BW, Cooke NE, Pike JW. 2008. Vitamin D-binding protein influences total circulating levels of 1,25-dihydroxyvitamin D3 but does not directly modulate the bioactive levels of the hormone in vivo. Endocrinology 149(7):3656–3667.

Zhao J, Tan BK, Marcelis S, Verstuyf A, Bouillon R. 1996. Enhancement of antiproliferative activity of 1alpha,25-dihydroxyvitamin D3 (analogs) by cytochrome P450 enzyme inhibitors is compound- and cell-type specific. J Steroid Biochem Mol Biol 57(3–4):197–202.