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Vitamin D3 polyunsaturated side-chain analogues (EB1089, GS1590) and the 20-epi-vitamin D3 analogue CB1393 suppress parathyroid hormone secretion and mRNA level in bovine parathyroid cells

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Abstract

Several vitamin D analogues, with reduced hypercalcemic and hyperphosphatemic toxicity at therapeutic dosages, are in clinical use for prevention and treatment of secondary hyperparathyroidism (HPT) in chronic renal failure. We have performed a first in vitro evaluation of five vitamin D analogues displaying less calcemic activity in normal rats, considerably more antiproliferative ability and higher transcription activation potential than 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), with the future prospects in mind to identify even more effective and less calcemic vitamin D analogues for treatment of HPT. The vitamin D analogues EB1089 and GS1590 have polyunsaturated side-chains, whereas HEP187, MC1598 and CB1393 display altered stereochemistry at carbon 20. In primary cultures of bovine parathyroid cells EB1089, GS1590, CB1393 and MC1598 as well as the comparative controls $1,25(OH)_2D_3$, 22-oxacalcitriol (OCT, maxacalcitol), 19-nor-$1,25(OH)_2D_2$ (paricalcitol) and $1\alpha(OH)D_2$ (doxercalciferol) significantly suppressed PTH secretion or reduced PTH mRNA level at 10^{-8}, 10^{-10}, and 10^{-11} M for all compounds except for MC1598 at the lowest concentration. The analogue HEP187 displayed no PTH suppressive activity. We conclude that the vitamin D analogues EB1089, GS1590 and CB1393 may be suitable for treatment of hyperparathyroidism secondary to uremia and that further evaluation in vivo should be considered.

Keywords: Vitamin D3; 1,25-Dihydroxyvitamin D_3; Hyperparathyroidism; Vitamin D_3 analogues

1. Introduction

The active metabolite of vitamin D_3, $1,25(OH)_2D_3$ and its synthetic analogues have potential therapeutic applications for treatment of several disorders due to their immunomodulatory, prodifferentiating and anti-proliferative activities. To reduce development of hypercalcemia and hyperphosphatemia, the major side effects of $1,25(OH)_2D_3$ treatment, synthetic vitamin D receptor (VDR) ligands with less toxicity are being developed for various medical applications. $1,25(OH)_2D_3$ controls growth of the parathyroid gland and suppresses parathyroid hormone (PTH) gene transcription and hormone secretion [1,2]. Hypocalcemia caused by vitamin D deficiency and hyperphosphatemia in renal failure predisposes to secondary HPT with oversecretion of PTH and stimulated growth of the parathyroid glands (hyperplasia), eventually leading to hypercalcemia [3,4]. Currently, four PTH suppressive vitamin D analogues [5,6] with less toxicity than $1,25(OH)_2D_3$ are being used and explored clinically for treatment of HPT secondary to uremia ($1\alpha(OH)D_2$ (doxercalciferol), 19-nor-$1,25(OH)_2D_2$ (paricalcitol), 22-oxacalcitriol (OCT, maxacalcitol), $1,25(OH)_2$-26,27F6 D_3 (falecalcitriol)). Results from comparative clinical studies in dialysis patients suggested a clinical advantage of 19-nor-$1,25(OH)_2D_2$ over $1,25(OH)_2D_3$ [7,8].

In an attempt to identify even more effective vitamin D analogues for treatment of secondary HPT we have performed a first in vitro evaluation of five analogues (EB1089 (seocalcitol), GS1590, CB1393, MC1598 and HEP187). Their effects on PTH secretion and PTH mRNA level were measured on primary cultures of bovine parathyroid cells. EB1089 is a well-characterized vitamin D analogue with potent antitumour activities, which is presently undergoing clinical investigations in patients with hepatocellular

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carcinomas [9,10]. GS1590 is a new vitamin D analogue, structurally related to EB1089, but with less calcemic activity. HEP187, CB1393 and MC1598 are all 20-epi-vitamin D analogues, with altered stereochemistry at carbon 20 in the side-chain. Of these, HEP187 is the best characterized so far, with ability to promote bone formation and increase bone strength in animal models of osteoporosis [11].

2. Materials and methods

2.1. Calcium metabolism in normal rats

Calcemic effects of the vitamin D analogues were assessed in LEW/mol female rats (130–170 g) receiving a standard laboratory diet containing 1% calcium and 0.75% phosphorus. Hypercalciuria was chosen as the most sensitive test parameter of vitamin D activity. The test compounds were administered orally, daily for 7 days. The rats received 1, 10 or 100 μg/kg of 22-oxacalcitriol; 0.1, 1.0 or 10 μg/kg of CB1393; 0.1, 0.5 or 1 μg/kg of EB1089; 0.1, 1 or 10 μg/kg of GS1590; 0.1, 0.5 or 1 μg/kg of HEP187; and 0.1, 1 or 10 μg/kg of MC1598. Control rats received the vehicle (propylene glycol) and each experiment included a group treated with $1,25(OH)_2D_3$ (0.5 μg/kg per day). Each group consisted of three rats. The rats were placed in metabolism cages and urine was collected daily. Calcium in urine was determined by complex formation with o-cresolphthalein. The urinary excretion of calcium was calculated from day 3 to 7 of the experiment (steady-state conditions) and serum calcium was measured at day 7. Mean urine calcium values caused by three different vitamin D analogue concentrations and the vehicle provided a dose-response curve, in which the analogue concentration was determined that would cause the same calcemic effect as $1,25(OH)_2D_3$ (0.5 μg/kg per day). The ratio between $1,25(OH)_2D_3$ (0.5 μg/kg per day) and this concentration provided the relative calcemic effect. The choice of the $1,25(OH)_2D_3$ dosage of 0.5 μg/kg per day was based on a full-scale dose-response experiment (0.01–10 μg/kg per day). The experiment was repeated two or three times depending on the analogue, except for 22-oxacalcitriol which was performed once (see Table 1).

2.2. MCF7 cell proliferation assay

MCF7 cells (7×10^3 cells per ml) were cultured in 48-well tissue culture plates and increasing concentrations of test compounds were added 2 h after seeding. After 5 days, the effect of the test compounds on the DNA synthesis was assessed by measuring the incorporation of [^{3}H]-thymidine for the last 4 h of culture. The IC_{50} values were calculated and inhibitory effects of the analogues were expressed relative $1,25(OH)_2D_3$.

2.3. VDR-binding assay

Fixed concentration of VDR protein from the intestinal epithelium of rachitic chickens (Amersham Biosciences) was incubated with ^{3}H-$1,25(OH)_2D_3$ (180 Ci/mmol, Amersham Biosciences) and increasing concentrations of $1,25(OH)_2D_3$ or the test compounds were added. After incubation for 60 min at 22 °C, bound and free ^{3}H-$1,25(OH)_2D_3$ were separated with dextran-coated charcoal. The samples were centrifuged, and the levels of receptor-bound ^{3}H-$1,25(OH)_2D_3$ contained in the supernatants were determined with a beta counter. The concentration resulting in 50% displacement of bound ^{3}H-$1,25(OH)_2D_3$ was calculated.

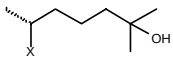
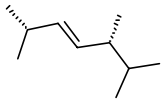
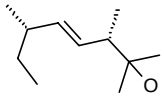
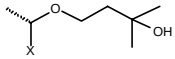
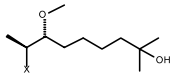
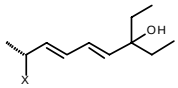
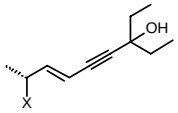
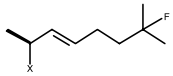
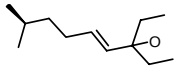
2.4. Transient transfection of MCF-7 cells

A reporter plasmid carrying the firefly luciferase gene under transcriptional control of the VDR was transiently transfected into MCF-7 breast cancer cells using DOTAP liposomal transfection reagent (Roche). The cells were seeded at a density of 3×10^4 cells per ml in 6-well Multi-Dishes and transfected on the 4th day when the cells were about 80% confluent. The transcription from the luciferase gene is regulated through a vitamin D responsive element of the DR3 type from the rat atrial natriuretic factor promoter [12]. The reporter contains four copies of the vitamin D responsive element linked to the thymidine kinase promoter (M.W. Madsen, unpublished). After transfection, cells were incubated with increasing concentrations of the various vitamin D compounds. The cells were harvested 24 h later and luciferase activity was determined and the EC_{50} were calculated. The effect of the analogues were expressed relative to $1,25(OH)_2D_3$.

2.5. Parathyroid cell culture

Bovine parathyroid glands were collected from adult cattle 15–20 min after slaughter. After removal of visible fat and connective tissue, the glands was minced with scissors. Cell suspensions were prepared by digestion in 1 mg/ml collagenase (Sigma, St Louis, Missouri, USA), 0.05 mg/ml DNase I, 1.5% bovine serum albumin and 1.25 mM Ca^{2+} as previously described [13]. After digestion in a shaking incubator for 120 min, the suspensions were filtered through nylon mesh (125 μm) and exposed to 1 mM EGTA in 25 mM HEPES buffer (pH 7.4) containing 142 mM NaCl and 6.7 mM KCl. Debris and dead cells were removed by centrifugation through 25 and 75% standard isotonic Percoll (Amersham Biosciences Europe, Germany). Cell viability, as determined by the Trypan blue exclusion test, exceeded 95%. Cells were placed in 6-well dishes, with 10^6 cells per well, and cultured for 4 h in DMEM/Ham's F-12, 1 mM total calcium, 4% fetal bovine serum, 15 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml insulin, 2 mM

Table 1
Characteristics of the vitamin D compounds included in the study

Compound	Side-chain structure	Calcemic activity relative to $1,25(OH)_2D_3$	Inhibition of proliferation in MCF7 relative to $1,25(OH)_2D_3$	VDR-binding relative to $1,25(OH)_2D_3$	VDR transcriptional activity relative to $1,25(OH)_2D_3$
$1,25(OH)_2D_3$		100%	1	100%	1
$1\alpha(OH)D_2$		Not determined	<0.3 ($n = 3$)	Not determined	0.6 ($n = 2$)
19-$norD_2$		Not determined	<0.3 ($n = 2$)	Not determined	0.1 ($n = 1$)
22-Oxacalcitriol (OCT)		30% ($n = 1$)	2 ($n = 4$)	10% ($n = 1$)	0.2 ($n = 2$)
CB1393		10% ($n = 2$)	153 ($n = 3$)	<5% ($n = 1$)	13 ($n = 3$)
EB1089		50% ($n = 3$)	109 ($n = 18$)	35% ($n = 3$)	92 ($n = 5$)
GS1590		16% ($n = 2$)	192 ($n = 1$)	43% ($n = 2$)	25 ($n = 5$)
HEP187		41% ($n = 2$)	67 ($n = 1$)	4% ($n = 1$)	10 ($n = 4$)
MC1598		17% ($n = 2$)	101 ($n = 3$)	50% ($n = 1$)	7 ($n = 2$)

n, Number of individual experiments. The mean values are shown. CB1393: 1,25-dihydroxy-22-methoxy-20-epi-24-bishomo-cholecalciferol; EB1089: 1,25-dihydroxy-24,26,27-trishomo-22(E),24(E)-diene-cholecalciferol; GS1590: 1,25-dihydroxy-24,26,27-trishomo-22(E)-ene-24-yne-cholecalciferol; HEP187: 1-hydroxy-25-fluoro-20-epi-24-homo-22(E)-ene-cholecalciferol; MC1598: 1,25-dihydroxy-20-epi-24,26,27-trishomo-24(E)-ene-cholecalciferol.

glutamine and 1% nonessential amino acids [14]. Medium was then replaced with the same as above, with the exception for 1 mg/ml bovine serum albumin instead of serum, addition of holo-transferrin to 5 μg/ml and 0.1% ethanol or vitamin D analogues. The serum-free medium was replenished once, 28 h before harvest. After 60 h of hormone exposure, the cells were tested for PTH secretion for 4 h, before medium was collected and the cells were harvested. The PTH protein concentration in the medium was measured with a radioimmunoassy kit (Peninsula Lab Inc., San Carlos, CA) and the PTH amount was related to total RNA extracted from each well. Experiments were done at least in triplicates. Parathyroid cells prepared and cultured as described here exhibit the inverse relationship between $[Ca^{2+}]_i$ and PTH secretion [15,16].

2.6. RNA extraction and cDNA synthesis

Total RNA was extracted from the cultured bovine parathyroid cells using TriZol reagent (Invitrogen Corp., Carlsbad, CA). The total RNA was treated with RQ1 Dnase I (Promega, Madison, WI) before further purification on Rneasy Mini kit columns (Qiagen, Hilden, Germany). One microgram of RNA was reverse transcribed to cDNA using the first strand cDNA Synthesis Kit (Amersham Biosciences).

2.7. Semi-quantitative RT–PCR analysis

Expression levels of bovine PTH mRNA in relation to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were semi-quantitatively determined by RT–PCR analysis. The number of PCR cycles that yielded measurable PCR products, avoiding saturating PCR levels, were used for each transcript. The following mRNA-specific PCR primers were used: PTH (forward): 5′-GACATGGCTAAAGTTATGAT; PTH (reverse): 5′-CAGCTTCTTACGCAGCCATTCTAC; GAPDH (forward): 5′-GGTCATCATCTCTGCACCTTC; GAPDH (reverse): 5′-CTTCTGGGTGGCAGTGATGGC. Expected sizes of the PCR products were 162 bp for bovine PTH and 205 bp for bovine GAPDH. Sequence analysis of the PCR fragments were undertaken on ABI 373A using ABI prism dye terminator cylce sequencing ready reaction kit (Applied Biosystems, New Jersey). The sequence revealed complete homology to the published bovine PTH mRNA sequence (*V00106*). The bovine GAPDH sequence showed

almost identity to the human counterpart with a few base changes. The PCR reactions contained 2 μl of cDNA (diluted 3×), 1 × PCR buffer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, for both PTH and GAPDH. Each PCR reaction for GAPDH contained 1.25 U of Platinum Taq polymerase (Invitrogen) and 0.4 pmol/μl of primers, whereas 1.25 U of Taq polymerase (Invitrogen) and 0.5 pmol/μl of primers were used for the amplification of bovine PTH. The PCR reaction parameters for PTH were 95 °C for 3 min followed by 95 °C for 20 s, 54 °C 20 s and 72 °C for 30 s repeated 24 cycles followed by extension at 72 °C for 7 min. Amplification parameters for GAPDH were similar to PTH, except for denaturation at 95 °C for 1 min, annealing at 58 and 72 °C for 20 s as extension in each cycle. Eight microliter of each PCR reaction were separated on 2.0% agarose gel stained with ethidium bromide. The intensity of each PCR fragment was quantified by molecular analysis software (Bio-Rad Lab., Richmond, CA). As negative control, water instead of cDNA, was subjected to PCR. No PCR products were obtained.

2.8. Statistical analysis

All statistical calculations were made with Stat View 5.0 (SAS Institute, Inc., Cary, NC). Values are presented as the mean ± S.E.M. and Student's unpaired *t*-test was used. $P < 0.05$ was considered significant.

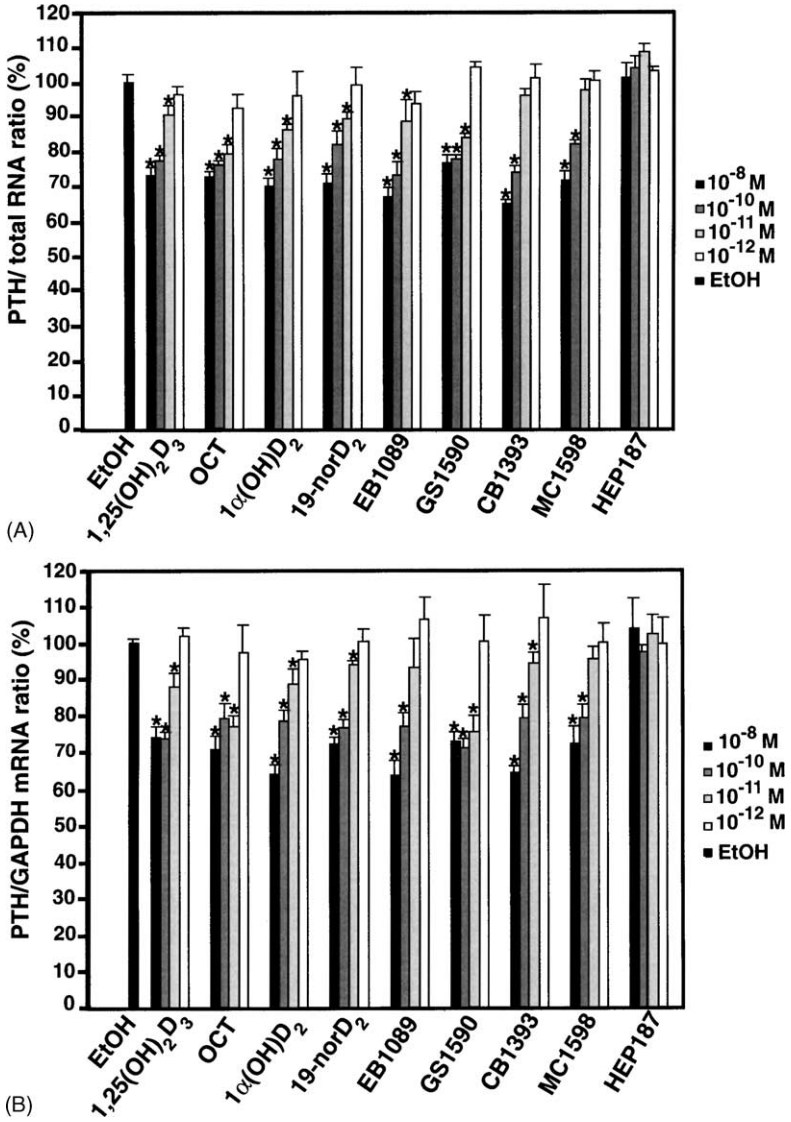


Fig. 1. Suppression of PTH (A) and PTH mRNA (B) in primary cultures of bovine parathyroid cells exposed to vehicle (ethanol), $1,25(OH)_2D_3$, 22-oxacalcitriol (OCT), $1\alpha(OH)D_2$, 19-nor-D_2, EB1089, GS1590, CB1393, MC1598, and HEP187. The mean ± S.E.M. is shown from at least two independent cell culture experiments performed in triplicates. Statistical significance was calculated using Student's unpaired *t*-test. Results that differ significantly ($P < 0.05$) from the vehicle control (100%) are indicated with asterisk.

3. Results and discussion

Characteristics of the vitamin D analogues EB1089, GS1590, CB1393, MC1598 and HEP187 in comparison to $1,25(OH)_2D_3$, $1\alpha(OH)D_2$, 19-nor-$1,25(OH)_2D_2$ and 22-oxacalcitriol are summarized in Table 1. EB1089, GS1590, CB1393, MC1598 and HEP187 were choosen based on their considerably less calcemic activity in rats as compared to $1,25(OH)_2D_3$, and on increased inhibition of cellular proliferation and increased VDR transactivation potential as compared to $1,25(OH)_2D_3$, $1\alpha(OH)D_2$, 19-nor-$1,25(OH)_2D_2$ and 22-oxacalcitriol. Primary bovine parathyroid cells were exposed to vehicle, $1,25(OH)_2D_3$, $1\alpha(OH)D_2$, 19-nor-$1,25(OH)_2D_2$, 22-oxacalcitriol and the five analogues for 60 h at concentrations of 10^{-8}, 10^{-10}, 10^{-11} and 10^{-12} M. After another 4 h of incubation in fresh medium, without vitamin D analogues, the media were collected and assayed for PTH. Cells were harvested from the same cultures and total RNA was prepared. The RNA was used as reference and for quantitative determination of PTH mRNA levels using RT–PCR. Fig. 1 shows the PTH suppressing potencies of the five analogues in comparison to $1,25(OH)_2D_3$, $1\alpha(OH)D_2$, 19-nor-$1,25(OH)_2D_2$ and 22-oxacalcitriol. EB1089, GS1590, CB1393, MC1598 but not HEP187 suppressed PTH secretion (Fig. 1A) and reduced PTH mRNA level (Fig. 1B). The analogues EB1089, GS1590, CB1393 and MC1598 as well as $1,25(OH)_2D_3$, 22-oxacalcitriol, 19-nor-$1,25(OH)_2D_2$ and $1\alpha(OH)D_2$ significantly ($P < 0.05$) suppressed PTH secretion or reduced PTH mRNA level by 23–36% at 10^{-8} M, 18–29% at 10^{-10} M and 5–24% at 10^{-11} M for all compounds except for MC1598 at the lowest concentration. No PTH suppressive effects were seen at 10^{-12} M. Overall, in this assay EB1089, GS1590, CB1393, and MC1598 showed a PTH inhibitory potentiality similar to $1,25(OH)_2D_3$, $1\alpha(OH)D_2$, 19-nor-$1,25(OH)_2D_2$ and 22-oxacalcitriol. Of all the analogues analyzed, only 22-oxacalcitriol and GS1590 suppressed PTH secretion or reduced PTH mRNA level by 20–30% also at 10^{-11} M, with no significant difference between the two analogues. The present study has identified several novel vitamin D analogues that fulfill primary criteria for possible treatment of secondary HPT to uremia. In addition to the reduced calcemic activity in rats of EB1089, GS1590 and CB1393 by 50, 16 and 10% relative to $1,25(OH)_2D_3$, respectively, excellent PTH suppressive effects were displayed in vitro. In addition, the increased inhibitory effects on proliferation of MCF7 cells by these analogues (Table 1) may also be beneficial for interfering with parathyroid hyperplasia associated with secondary HPT. Unfortunately, we had to refrain from cellular proliferation analysis on primary bovine parathyroid cells, of the analogues analyzed here, as this assay [17] does not work in our hands. Of the five novel vitamin D analogues tested here four displayed relatively high VDR transcription activation potential in MCF7 cells (Table 1) in addition to transcription reducing potential of the PTH gene in parathyroid cells (Fig. 1B). HEP187 on the other hand strongly activated transcription through VDR, but failed to suppress PTH secretion or reduce PTH mRNA level. This most likely reflects inability of HEP187 to induce a functional parathyroid ligand/VDR/co-factor/DNA complex conformation that negatively regulates PTH gene transcription [18].

In summary, EB1089, GS1590 and CB1393 effectively suppressed PTH in vitro with less calcemic activities in normal rats and they should be further evaluated in uremic rats and in clinical studies with hope for effective inhibition of both PTH secretion and parathyroid hyperplasia in uremia together with reduced risks of hypercalcemia and hyperphosphatemia.

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References

[1] G. Jones, S.A. Strugnell, H.F. DeLuca, Current understanding of the molecular actions of vitamin D, Physiol. Rev. 78 (1998) 1193–1231.
[2] A.J. Brown, A. Dusso, E. Slatopolsky, Vitamin D, Am. J. Physiol. 277 (1999) F157–F175.
[3] S.J. Marx, Hyperparathyroid and hypoparathyroid disorders, N. Engl. J. Med. 343 (2000) 1863–1875.
[4] J. Silver, R. Kilav, A. Sela-Brown, T. Naveh-Many, Molecular mechanisms of secondary hyperparathyroidism, Pediatr. Nephrol. 14 (2000) 626–628.
[5] T. Akizawa, K. Shiizaki, et al., New strategies for the treatment of secondary hyperparathyroidism, Am. J. Kidney Dis. 41 (2003) S100–S103.
[6] E. Slatopolsky, J. Finch, A. Brown, New vitamin D analogs, Kidney Int. 63 (2003) S83–S87.
[7] S.M. Sprague, F. Llach, M. Amdahl, C. Taccetta, D. Batlle, Paricalcitol versus calcitriol in the treatment of secondary hyperparathyroidism, Kidney Int. 63 (2003) 1483–1490.
[8] M. Teng, M. Wolf, E. Lowrie, N. Ofsthun, M. Lazarus, R. Thadhani, Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy, N. Engl. J. Med. 349 (2003) 446–456.
[9] C. Mørk-Hansen, K.J. Hamberg, E. Binderup, L. Binderup, Seocalcitol (EB1089): a vitamin D analogue of anti-cancer potential. Background, design, synthesis, pre-clinical and clinical evaluation, Curr. Pharmaceut. Des. 6 (2000) 803–828.
[10] K. Dalhoff, J. Dancey, L. Astrup, T. Skovsgaard, J.K. Hamberg, F.J. Lofts, O. Rosmorduc, S. Erlinger, J.B. Hansen, W.P. Steward, T. Skov, F. Burcharth, T.R.J. Evans, A phase II study of the vitamin D analogue Seocalcitol in patients with inoperable hepatocellular carcinoma (HCC), Br. J. Cancer 89 (2003) 252–257.
[11] M. Tranholm, P.K. Holm, C. Mørk Hansen, S. Ryhänen, S. Väisänen, L. Binderup, J. Skovhus Thomsen, P. Mäenpää, L. Mosekilde, In vitro and in vivo effects of a 1,25-dihydroxyvitamin D3 analog (HEP187) on bone, ASBMR, 22nd Annual meetings, Toronto, Canada, September 2000.
[12] J.-P. Kahlen, C. Carlberg, Functional characterization of a 1,25-dihydroxyvitamin D_3 receptor binding site found in the rat atrial natriuretic factor promoter, Biochem. Biophys. Res. Com. 218 (1996) 882–886.

[13] C. Rudberg, L. Grimelius, H. Johansson, et al., Alterations in density, morphology and parathyroid hormone release of dispersed parathyroid cells from patients with parathyroidism, Acta. Path. Microbiol. Immunol. Cand. Sect. A 94 (1986) 253–261.
[14] P.N. MacDonald, C. Ritter, A.J. Brown, et al., Retionic acid suppresses parathyroid hormone (PTH) secretion and preproPTH mRNA levels in bovine cell culture, J. Clin. Invest. 93 (1994) 725–730.
[15] R. Larsson, C. Wallfelt, H. Abrahamsson, E. Gylfe, S. Ljunghall, J. Rastad, P. Rorsman, L. Wide, G. Åkerström, Defective regulation of the cytosolic Ca^{2+} activity in parathyroid cells from patients with hyperparathyroidism, Biosci. Rep. 4 (1984) 909–915.
[16] C. Wallfelt, E. Lindh, R. Larsson, H. Johansson, J. Rastad, G. Åkerström, E. Gylfe, Kinetic evidence for cytoplasmic calcium as an inhibitory messenger in parathyroid hormone release, Biochim. Biophys. Acta 969 (1988) 257–262.
[17] Y. Imanishi, M. Inaba, H. Seki, H. Koyama, Y. Nishizawa, H. Morii, S. Otani, Increased biological potency of hexafluorinated analogs of 1,25-dihydroxyvitamin D3 on bovine parathyroid cells, J. Steroid. Biochem. Mol. Biol. 70 (1999) 243–248.
[18] C. Carlberg, Molecular basis of the selective activity of vitamin D analogues, J. Cell Biochem. 88 (2003) 274–281.