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Vitamin D Hormone Inhibits Osteoclastogenesis In Vivo by Decreasing the Pool of Osteoclast Precursors in Bone Marrow

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ABSTRACT

Previous observations that vitamin D hormone induces the expression of the receptor activator of nuclear factor κB (NF-κB) ligand (RANKL), thereby stimulating osteoclastogenesis in vitro, led to the widespread belief that 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] is a bone-resorbing hormone. Here, we show that alfacalcidol, a prodrug metabolized to 1α,25$(OH)_2D_3$, suppresses bone resorption at pharmacologic doses that maintain normocalcemia in an ovariectomized (OVX) mouse model of osteoporosis. Treatment of OVX mice with pharmacologic doses of alfacalcidol does not increase RANKL expression, whereas toxic doses that cause hypercalcemia markedly reduce the expression of RANKL. When bone marrow (BM) cells from OVX mice were cultured with sufficient amounts of macrophage colony-stimulating factor (M-CSF) and RANKL, osteoclastogenic activity was higher than in sham mice. Marrow cultures from alfacalcidol- or estrogen-treated OVX mice showed significantly less osteoclastogenic potential compared with those from vehicle-treated OVX mice, suggesting that the pool of osteoclast progenitors in the marrow of vitamin D–treated mice as well as estrogen-treated mice was decreased. Frequency analysis showed that the number of osteoclast progenitors in bone marrow was increased by OVX and decreased by in vivo treatment with alfacalcidol or estrogen. We conclude that the pharmacologic action of active vitamin D in vivo is to decrease the pool of osteoclast progenitors in BM, thereby inhibiting bone resorption. Because of its unusual activity of maintaining bone formation while suppressing bone resorption, in contrast to estrogens that depress both processes, vitamin D hormone and its bone-selective analogs may be useful for the management of osteoporosis. (J Bone Miner Res 2002;17:622–629)

Key words: vitamin D, alfacalcidol, osteoporosis, bone resorption, osteoclasts, receptor activator of nuclear factor κB ligand, ovariectomized mice

INTRODUCTION

VITAMIN D receptor (VDR) liganded with 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], the active and hormonal form of vitamin D, plays a critical role in calcium and bone metabolism through its action in the intestine, bone, kidney, and parathyroid gland.[1,2] The importance of the VDR, especially during the maturation stage of bone, can be inferred from the consequences of functional defects in it; these include hypocalcemia, secondary hyperparathyroidism, and impaired mineralization, which are the phenotypes shown in the human vitamin D–resistant rickets syndrome due to point mutations in the VDR gene[3,4] and also in VDR knockout mice.[5,6] In contrast, the physiological importance of

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VDR in normal bone remodeling after skeletal maturation remains unclear.

Age-related decline in VDR function is thought to increase the risk of developing osteoporosis, a systemic disease characterized by excessive bone resorption with relative deficiency of bone formation.[7,8] Thus, $1\alpha,25(OH)_2D_3$ and its prodrug 1α-hydroxyvitamin D_3 [$1\alpha(OH)D_3$; or alfacalcidol] have been used in some countries for the treatment of osteoporosis.[9] However, how the hormonal form of vitamin D acts in bone to counter the abnormal remodeling process in osteoporosis remains to be clarified.

Generally, it is recognized that the effect of $1\alpha,25(OH)_2D_3$ on bone is an indirect one, mediated by stimulation of calcium absorption from the intestine and resultant suppression of parathyroid hormone (PTH),[7] and how $1\alpha,25(OH)_2D_3$ modulates bone cell functions under vitamin D–replete conditions remains relatively unclear. At the cellular level, it has been established that $1\alpha,25(OH)_2D_3$ induces the expression of the receptor activator of nuclear factor κB (NF-κB) ligand (RANKL) in bone marrow (BM) stromal cells,[10] which is essential for the differentiation of bone-resorbing osteoclasts.[11,12] This in vitro property of $1\alpha,25(OH)_2D_3$ is consistent with the well-known activity of $1\alpha,25(OH)_2D_3$ in stimulating osteoclastic bone resorption in organ cultures[13] but is inconsistent with its therapeutic use in osteoporosis.

Based on a series of in vivo experiments using an ovariectomized (OVX) rat model of estrogen-deficient osteoporosis, we have shown that contrary to the in vitro activity of $1\alpha,25(OH)_2D_3$, active vitamin D inhibits osteoclastic bone resorption as potently as estrogen[14] and that this anticatabolic effect is at least in part mediated through a direct effect on the skeletal tissue independently of the calcium flux from the intestine and the PTH levels.[15] To gain further insight into the mechanism by which active vitamin D inhibits bone resorption, we used murine marrow cultures for osteoclastogenesis using BM cells from OVX mice treated in vivo with the drugs. The results indicated that the hormonal form of vitamin D, at pharmacologic doses that maintain normocalcemia, does not induce RANKL in bone but inhibits bone resorption by decreasing the pool size of osteoclast precursors in BM.

MATERIALS AND METHODS

Reagents

Alfacalcidol [$1\alpha(OH)D_3$] was synthesized at Chugai Pharmaceutical Co., Ltd. (Shizuoka, Japan), dissolved in a vehicle (medium-chain triglyceride [MCT]), and diluted to a given concentration. 17β-Estradiol (E_2), purchased from Sigma (St. Louis, USA), was dissolved in corn oil (Sigma) and diluted to a given concentration. The stock solutions were protected from light and stored at 4°C. Macrophage colony-stimulating factor (M-CSF) was purchased from R & D Systems, Inc. (Minneapolis, USA), and soluble RANKL was a gift from Snow-Brand Milk Product Co. (Tochigi, Japan)

Animals

Seven-week-old female *ddy* mice were purchased from Nippon SLC. (Shizuoka, Japan) and acclimated for 1 week under standard laboratory conditions at 24 $\pm$ 2°C and 50–60% humidity. The mice were allowed free access to tap water and commercial standard rodent chow (CE-2) containing 1.13% calcium, 1.00% phosphate, and 2.0 IU/g of vitamin D_3 (Clea Japan, Inc., Shizuoka, Japan).

Experimental design

Sham-operated and OVX mice were used. The OVX mice were divided randomly into several groups after surgery. In the sham and OVX control groups, mice received the vehicle (MCT) orally at a dose of 1 ml/kg body weight (BW) five times a week. Alfacalcidol was given orally at 0.05 μg/kg and 0.2 μg/kg BW five times a week, and E_2 was injected subcutaneously at 20 μg/kg BW five times a week. These animal studies were carried out in accordance with the ethical guidelines for animal care at Chugai Pharmaceutical Co., Ltd., and the National Institute for Longevity Sciences, and the experimental protocols were approved by the animal care committees of both institutions.

Serum and urine biochemistry

Urine was collected during 24 h of fasting, and blood was drawn from the abdominal aorta under ether anesthesia. Blood and urine samples were centrifuged to obtain the supernatants, which were stored at −20°C until the assay. Serum calcium (Ca) and inorganic phosphorus (P_i) concentrations were measured with an autoanalyzer (7070; Hitachi, Tokyo, Japan). The serum osteocalcin concentration was measured with the Mouse Osteocalcin IRMA Kit (Immutopics, Inc., San Clemente, CA, USA). Urinary Ca, P_i, and creatinine (Cr) were measured with an autoanalyzer. Urinary deoxypyridinoline (D-Pyr) was measured using a PYRILINKS-D assay kit (Metra Biosystems, Inc., Mountain View, CA, USA), and the data were corrected for urinary Cr concentrations.

Bone histomorphometry

The distal femora were fixed in ice-cold 70% ethanol. After dehydration with 2-propanol, the samples were embedded in glycol methacrylate (Wako, Osaka, Japan). Polymerization was performed at 4°C. Specimens were sectioned in the center to yield 3-μm undecalcified sections, which were then stained for tartrate-resistant acid phosphatase (TRAP). The image of the specimen, observed under a microscope and recorded with a video camera (DK-3000; Hitachi), was processed using a plotter (Cosmozone 1SA; Nikon, Tokyo, Japan) to measure the primary parameters, bone surface (BS, μm) and osteoclast number (N.Oc). From these primary parameters, the N.Oc per millimeter of trabecular surface (N.Oc/B.S./mm) was calculated. Osteoclasts were identified as $TRAP^+$ multinucleated cells that form resorption lacunae at BS. Nomenclature, symbols, and units

TABLE 1. EFFECTS OF ACTIVE VITAMIN D AND ESTROGEN ON BIOCHEMICAL PARAMETERS IN OVX MICE

			Serum			*Urine*	
Operation	*Treatment*	*Dose (μg/kg)*	*Calcium (mg/dl)*	*Phosphate (mg/dl)*	*Osteocalcin (ng/ml)*	*Calcium/Cr*	*D-Pyr/Cr (nM/mM)*
Sham	Vehicle		9.2 ± 0.6	7.7 ± 0.7	152.3 ± 5.6	0.103 ± 0.040*	31.77 ± 2.58†
OVX	Vehicle		9.5 ± 0.2	7.8 ± 0.9	179.6 ± 27.6	0.186 ± 0.071	43.62 ± 7.92
OVX	$1\alpha(OH)D_3$	0.05	9.2 ± 0.3	8.1 ± 0.9	158.0 ± 16.5	0.177 ± 0.064	32.56 ± 6.45*
OVX	$1\alpha(OH)D_3$	0.2	9.6 ± 0.5	8.8 ± 1.4	174.5 ± 28.2	0.629 ± 0.574	31.56 ± 5.36†
OVX	E_2	20	9.7 ± 0.6	8.2 ± 1.0	132.1 ± 16.7†	0.179 ± 0.042	34.43 ± 4.99*

Data represent mean ± SEM ($n = 6 \sim 8$).
* $p < 0.05$; † $p < 0.01$ versus OVX group.

used in this study are those described in the Report of the American Society for Bone and Mineral Research Nomenclature Committee.

Three-dimensional analysis of bone by microcomputed tomography

The microcomputed tomography (micro-CT) apparatus (μCT20) and the analysis software used in this study were provided by Scanco Medical AG (Bassersdorf, Switzerland).[16] The whole L5 bodies of the OVX mice and alfacalcidol- or estrogen-treated OVX mice were scanned dorsoventrally into 200 slices, each of which was 8 μm thick. On the original three-dimensional (3D) image, morphometric indices were determined directly from the binarized volume of interest (VOI). The total tissue volume (TV) was the volume of the whole sample examined. Then, the values of the trabecular bone volume fraction (BV/TV) and mean trabecular thickness (Tb.Th) were determined from the local thickness at each voxel representing bone.[17] Using this technique, thickness can be estimated without a model assumption. Trabecular separation (Tb.Sp) was calculated by applying the same technique as used for the direct thickness calculation to the nonbone parts of the 3D image.

In vitro osteoclastogenesis assay

OVX and drug-treated mice were killed by cervical dislocation, and the tibia and femur were removed and separated from the adhering soft tissues. The bone ends were cut off with a scalpel, and BM cells were collected by flushing with α-modified essential medium (α-MEM) containing 10% fetal calf serum (FCS). Sorted BM cells were cultured in α-MEM/10% FCS in the presence of M-CSF (5 ng/ml) and soluble RANKL (50 ng/ml). After 4 days, TRAP solution assay was performed. In the TRAP solution assay, enzyme activity was examined by conversion of α-naphthyl phosphate to α-naphthol in the presence of 20 mM of tartrate solution in each well. Absorbance was measured at 405 nm using a microplate reader (Model 550; Bio-Rad Laboratories, Hercules, CA, USA).

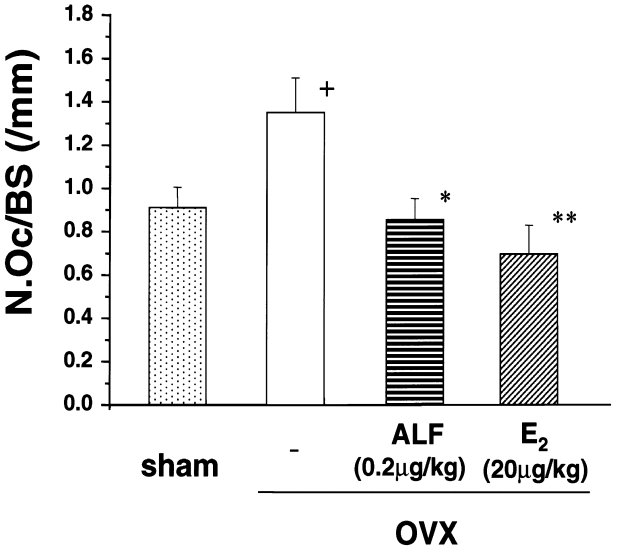

FIG. 1. Active vitamin D decreases N.Oc in OVX mice. OVX *ddy* mice (8 weeks old) were given alfacalcidol (ALF) orally (0.2 μg/kg BW) or E_2 subcutaneously (20 μg/kg BW) five times a week for 2 weeks, and N.Oc/BS at the distal femur was determined as described in Materials and Methods section. Data are expressed as mean ± SEM ($n = 7$). ** $p < 0.01$, * $p < 0.05$ versus the OVX-vehicle group, and $^{+}p < 0.05$ versus sham group, using Dunnett's multiple test.

For frequency analysis of osteoclast precursors, 1×10^4 ST2 cells were seeded into each well on 96-well plates and cultured overnight in α-MEM/10% FCS. One, 15, and 50 cells (as calculated by limiting dilution) of BM cells diluted with culture medium were then seeded onto the ST2 cell layer in each well. Cultures were maintained in α-MEM/10% FCS with 10^{-8} M of $1\alpha,25(OH)_2D_3$ and 10^{-7} M of dexamethasone for 7 days followed by fixation and staining for TRAP activity using a leukocyte acid phosphatase kit (Sigma). A well containing $TRAP^+$ multinucleated cells was judged to be osteoclast-positive.

Northern blot analysis

Total RNA was isolated from mice tibias using Trizol reagent (Life Technologies, Inc., Rockville, MD, USA).

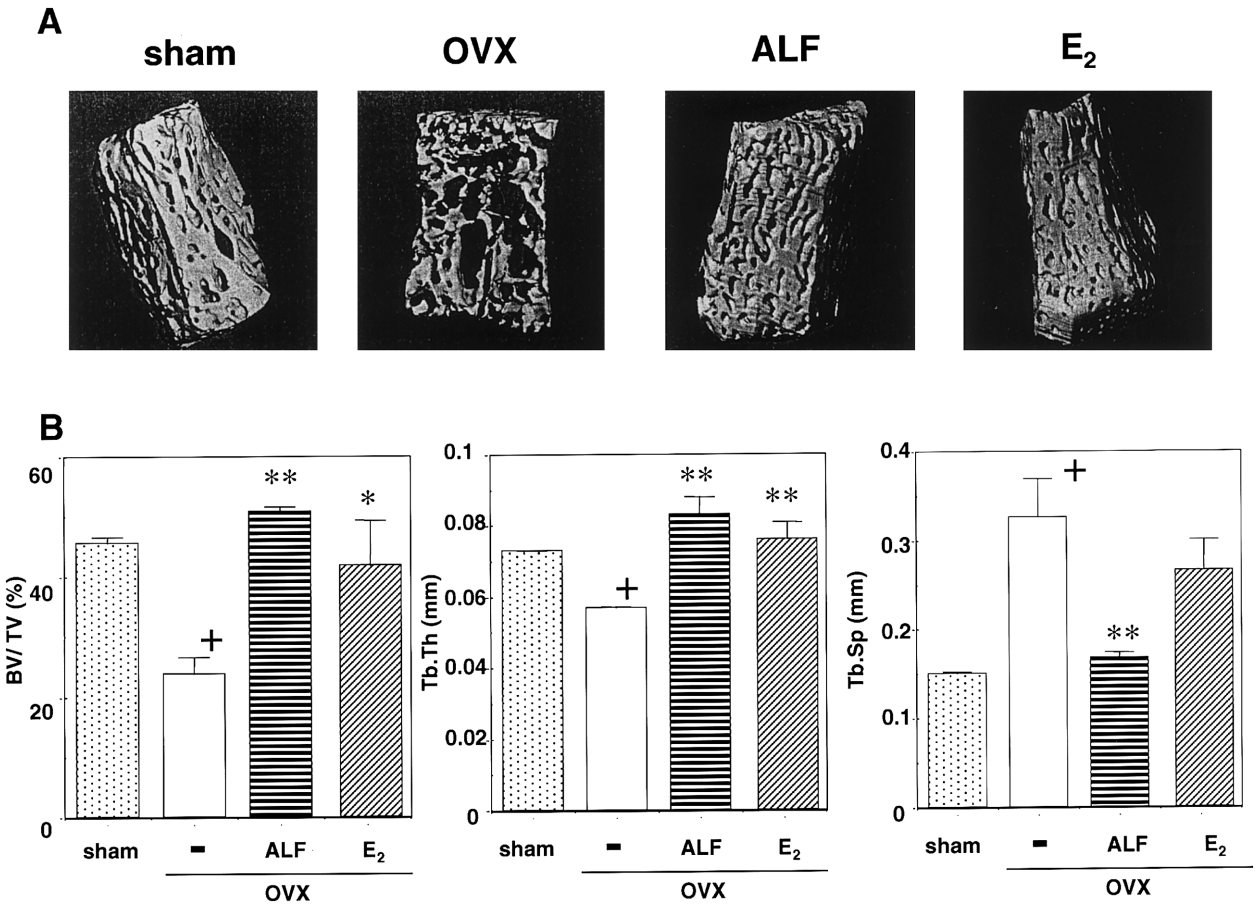

FIG. 2. Active vitamin D increases bone mass in OVX mice. (A) 3D trabecular bone architecture of the fifth lumbar vertebra in OVX and in alfacalcidol- or estrogen-treated OVX mice. OVX *ddy* mice (8 weeks old) were given alfacalcidol (ALF) orally (0.2 μg/kg BW) or E_2 subcutaneously (20 μg/kg BW) five times a week for 1 month, and the fifth lumbar vertebra was analyzed 3D using a micro-CT system, as described in the Materials and Methods section. Note that treatment with ALF or estrogen markedly increased trabecular bone in OVX mice. (B) Bone morphometric parameters using micro-CT shown in panel A. BV/TV, Tb.Th, and Tb.Sp were determined as described in the Materials and Methods section. Data are expressed as mean ± SEM ($n = 6–8$). $^{**}p < 0.01$, $^{*}p < 0.05$ versus the OVX-vehicle group, and $^{+}p < 0.05$ versus sham group, using Dunnett's multiple test.

Ten micrograms of RNA was glyoxalated, run on agarose gel, and transferred to GeneScreen Plus nylon membrane (NEN Life Science Product, Boston, MA, USA). Mouse RANKL, OPG, and mouse elongation factor 1 (EF1) complementary DNA (cDNA) were amplified by polymerase chain reaction (PCR) with oligonucleotide primers. EF1 messenger RNA (mRNA) served as an internal control in Northern blot analysis. PCR products were subcloned into pGEM-T (Promega, Madison, USA), and the cDNA probe was labeled with [α-^{32}P]deoxycytosine triphosphate (dCTP; NEN Life Science Product) by the random primer method (Amersham Pharmacia Biotech, Buckinghamshire, UK). Prehybridization and hybridization were carried out in Express-Hyb (Clontech, Palo Alto, CA, USA), and the filters were washed after hybridization, as recommended by the suppliers. Then, the filters were exposed to X-ray film or to the imaging plate of the Fujix Bio-Imaging Analyzer BAS2500 (Fuji Photo Film Co., Tokyo, Japan).

Statistical analysis

Data were expressed as the mean ± SEM. Statistical analysis was carried out by analysis of variance (ANOVA) using the Statistic Analysis System software (SAS Institute, Inc., Cary, NC, USA). The significances of differences were determined using Dunnett's multiple test (for comparison with OVX-vehicle group) or the Tukey-Kramer test. A value of $p < 0.05$ was considered significant.

RESULTS

Active vitamin D increases bone mass through inhibition of osteoclastic bone resorption in OVX mice

Previously, we have shown that active vitamin D is effective in preventing and treating osteoporosis caused by estrogen deficiency by suppressing osteoclastic bone resorp-

tion in an OVX rat model.[14] First, we examined whether alfacalcidol also suppresses osteoclastic bone resorption in OVX mice. As shown in Table 1, oral treatment with alfacalcidol at 0.05 μg/kg and 0.2 μg/kg BW decreased urinary D-Pyr excretion, which was elevated after OVX, and the magnitude of the effect was comparable with that of E_2. The results of histomorphometry indicate that treatment of OVX mice with alfacalcidol or E_2 decreased the number of osteoclasts at the distal femur (Fig. 1). Serum osteocalcin concentration, a marker of bone formation, increased after OVX and remained elevated after treatment with alfacalcidol, and estrogen replacement lowered serum osteocalcin levels significantly to within the sham range (Table 1). These results are consistent with our concept that estrogens depress bone turnover by inhibiting both bone resorption and formation, whereas active vitamin D suppresses bone resorption while maintaining bone formation.[14] At the small doses used in this study alfacalcidol did not raise the serum calcium concentration (Table 1).

Three-dimensional trabecular structure images obtained by micro-CT showed that bone mass, which substantially decreased after OVX, was markedly increased by treatment with alfacalcidol or estrogen (Fig. 2A). Alfacalcidol or estrogen increased BV/TV and Tb.Th to the sham levels (Fig. 2B). Tb.Sp, markedly increased by OVX, was significantly decreased by alfacalcidol treatment (Fig. 2B). Collectively, these results indicate that active vitamin D, at pharmacologic doses that do not cause hypercalcemia, inhibits osteoclastic bone resorption in vivo and thereby exerts therapeutic effects in estrogen-deficient osteoporosis.

Active vitamin D inhibits osteoclastogenesis in vivo by depleting osteoclast precursors

We then used murine marrow cultures for osteoclastogenesis to explore the mechanism by which vitamin D hormone suppresses osteoclastic bone resorption. BM cells, obtained from mice treated in vivo with alfacalcidol or estrogen after OVX, were cultured in the presence of M-CSF and RANKL, and their osteoclastogenic potential was assessed by TRAP staining. As shown in Fig. 3, osteoclastogenic activity was increased in marrow cultures from OVX mice, and this was significantly and dose-dependently suppressed by in vivo treatment with alfacalcidol as well as by estrogen. Because the osteoclastogenesis-supporting compounds M-CSF and RANKL were present in excess in these cultures and, therefore, were not rate-limiting, these results suggest that the pool of osteoclast progenitors in the marrow of vitamin D–treated mice may have been decreased.

To substantiate this concept, frequency analysis was performed. Theoretically, single marrow cells from OVX and drug-treated mice were cultured on the stromal layer of ST2 cells in the presence of $1\alpha,25(OH)_2D_3$, and the number of osteoclast progenitors originally present in the marrow was assessed by determining the number of wells with $TRAP^+$ osteoclasts (not the number of $TRAP^+$ osteoclasts formed). As shown in Fig. 4, the number of osteoclast progenitors was increased in the BM of OVX mice and was lowered by in vivo treatment with alfacalcidol or estrogen, showing that

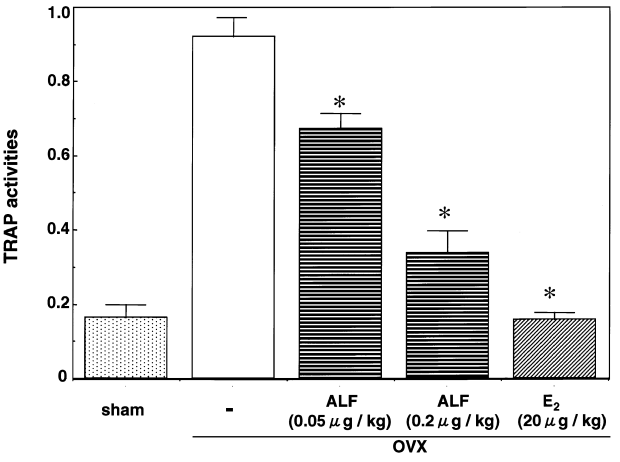

FIG. 3. Treatment in vivo with active vitamin D decreases the osteoclastogenic potential of in vitro marrow cultures. BM cells, obtained from mice treated in vivo with alfacalcidol (ALF; at 0.05 μg/kg and 0.2 μg/kg BW) or E_2 (at 20 μg/kg BW) after OVX, were cultured in the presence of M-CSF (5 ng/ml) and soluble RANKL (50 ng/ml), and their osteoclastogenic potential was assessed by TRAP assay as described in the Materials and Methods section. *$p < 0.001$ (compared with OVX-vehicle group using Dunnett's multiple test). Values represent mean ± SEM (n = 6–8).

active vitamin D decreases the pool of osteoclast progenitors in BM in vivo.

Active vitamin D suppresses the expression of RANKL mRNA in vivo at suprapharmacologic doses

The findings that vitamin D hormone suppresses osteoclastic bone resorption in vivo in OVX rats[14] and mice (indicated in this study) contradict with the long-held belief that $1\alpha,25(OH)_2D_3$ induces the expression of osteoclastogenesis-supporting RANKL[12] and is a bone-resorbing hormone.[13] To address this discrepancy, we examined the effects of in vivo treatment with alfacalcidol on RANKL expression in bone. As shown in Fig. 5A, Northern blot analysis revealed that RANKL mRNA expression in the whole femur did not change substantially after OVX or treatment of OVX animals with pharmacologic doses of alfacalcidol or E_2. When RNA from the diaphysis and metaphysis was analyzed separately, we did not observe any significant alteration in RANKL mRNA level among experimental groups at either site (data not shown). OPG mRNA was undetectable in the diaphysis and abundantly expressed in the metaphysis, again with no change between sham and OVX or OVX and drug-treated groups (data not shown).

Interestingly, superpharmacologic or toxic doses of alfacalcidol that caused hypercalcemia substantially reduced the expression of RANKL mRNA at the femur (Fig. 5B).

DISCUSSION

The previous observations that vitamin D hormone induces the expression of RANKL in stromal cells,[12] thereby

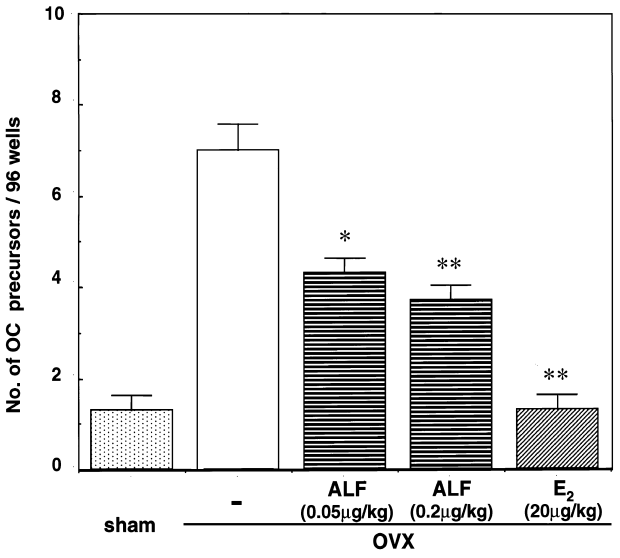

FIG. 4. Treatment with active vitamin D in vivo decreases the number of osteoclast precursors in BM. Single marrow cells (as calculated by limiting dilution) from OVX and alfacalcidol- (ALF; at 0.05 μg/kg and 0.2 μg/kg BW) or E_2 (at 20 μg/kg BW)-treated mice were cultured on the stromal layer of ST-2 cells in the presence of $1\alpha,25(OH)_2D_3$ (10^{-8} M) and dexamethasone (10^{-7} M), and the number of osteoclast progenitors originally present in the marrow was determined from the number of wells with $TRAP^+$ osteoclasts. $*p < 0.01$ and $**p < 0.001$ (vs. the OVX-vehicle group) by the Tukey-Kramer test.

stimulating osteoclastogenesis in vitro,[10] and that $1\alpha,25(OH)_2D_3$ stimulates bone resorption in an organ culture system[13] led to the widespread belief that $1\alpha,25(OH)_2D_3$ is a bone-resorbing hormone.[10] Our results indicate that active vitamin D, at pharmacologic doses that maintain normocalcemia, does not increase RANKL expression in vivo, whereas toxic doses of active vitamin D that cause hypercalcemia markedly reduce the expression of RANKL. These results suggest that vitamin D hormone causes hypercalcemia primarily by increased calcium absorption from the intestine, and hypercalcemia in turn may reduce the expression of RANKL as a compensatory mechanism.

This study suggests that the main pharmacologic action of vitamin D hormone in vivo, at least in a high bone turnover state such as estrogen deficiency, is to suppress osteoclastic bone resorption. Based on our previous findings that the therapeutic effects of vitamin D hormone on bone take place in parathyroidectomized animals,[18,19] it is suggested that at least part of the skeletal action of active vitamin D can take place independently of serum calcium and PTH levels, pointing to the direct action of vitamin D on bone. Furthermore, these data provide an important clue as to how vitamin D hormone acts in bone, not by suppressing the osteoclastogenesis-supporting microenvironment ("soil"), represented by RANKL, but by decreasing the number of osteoclast progenitors, that is, "seeds" of osteoclasts, in BM. In this respect, it is interesting to note that vitamin D deprivation in growing pigs is associated with an enlarged pool of osteoclasts,[20] a mirror image of the situation in our vitamin D–treated mice. It also is noted that estrogen deficiency is not associated with substantial up-regulation of RANKL in bone, at least at the RNA level (Fig. 5A), but with an enlarged pool of osteoclastogenic precursor cells (Fig. 4). Thus, the mechanisms by which the turnover of osteoclast progenitors is determined in BM under various

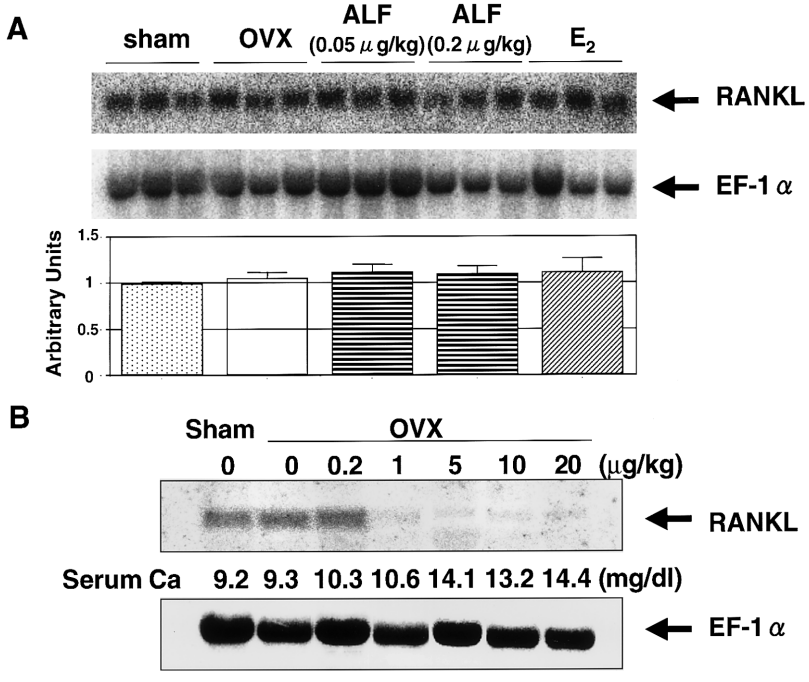

FIG. 5. The effects of active vitamin D in vivo on RANKL mRNA expression in the bone of OVX mice. (A) OVX *ddy* mice (8 weeks old) were given alfacalcidol (ALF) orally (at 0.05 μg/kg and 0.2 μg/kg BW) or E_2 subcutaneously (at 20 μg/kg BW) five times a week for 2 weeks, and RANKL mRNA expression in the whole femur was determined by Northern blot analysis. EF1α mRNA was used as an internal control. The bars represent mean $\pm$ SEM of RANKL mRNA level corrected for EF1α mRNA ($n = 3$ for each group). There was no statistical significance between the groups. (B) OVX mice were treated with the indicated doses (0.2, 1, 5, 10, and 20 μg/kg BW) of ALF for 2 weeks, and RANKL mRNA expression in the whole femur was determined by Northern blot analysis. The figures below the RANKL session indicate the serum calcium concentrations of the respective animals in milligrams per deciliter.

conditions and by which vitamin D hormone modulates the pool size may have important implications not only for understanding the physiology (e.g., development and aging) and pathology (e.g., estrogen deficiency) of the skeletal system, but also in drug development targeting these progenitor cells. Currently, we are analyzing the population enriched in osteoclast precursors such as c-Kit^{+}, Mac-1^{low}, and c-Fms^{+} cells[21,22] in the BM of drug-treated animals.

The molecular mechanism by which vitamin D hormone decreases the pool size of hematopoietic progenitors undergoing differentiation to osteoclasts remains unknown at present and requires further investigation. During the preparation of this study, Shevde et al. reported that estrogen inhibits M-CSF/RANKL-induced osteoclast differentiation, probably by interfering with c-Jun activity in osteoclast precursors.[23] In view of the tumor necrosis factor (TNF) receptor–associated factor (TRAF), NF-κB and c-Jun N-terminal kinase (JNK)/activator protein 1 (AP-1) pathways having been shown to represent essential signaling molecules acting downstream of RANKL binding to RANK on osteoclast precursors,[10] it is tempting to speculate that vitamin D hormone, like estrogens, interferes with one of these signaling pathways. Alternatively, because active vitamin D has been shown to induce the expression of cyclin-dependent kinase inhibitors such as p21^{Cip1} and p27^{Kip1},[24] and because p27^{Kip1} has been implicated as a factor determining the pool size of hematopoietic progenitors,[25] it is possible that the vitamin D hormone regulates the pool of osteoclast progenitors by modulating the activity of these cell cycle regulators.

In conclusion, we have established, using an OVX mouse model of osteoporosis and in vitro murine marrow cultures for osteoclastogenesis, that the pharmacologic action of active vitamin D in vivo is to decrease the pool of osteoclast progenitors in BM, thereby inhibiting bone resorption. In view of the unusual activity of the vitamin D hormone (it maintains bone formation while suppressing bone resorption,[18,19] unlike estrogens, which depress both processes), active vitamin D may provide a tool for designing useful VDR-based drugs for the management of osteoporosis.

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