Journal of Cellular Biochemistry 88:259–266 (2003)

Vitamin D and Bone

Tatsuo Suda,[1]* Yutaka Ueno,[2,3] Katsuyuki Fujii,[2] and Toshimasa Shinki[3]

[1]Research Center for Genomic Medicine, Saitama Medical School, Saitama 350-1241, Japan
[2]Department of Orthopaedic Surgery, Jikei University School of Medicine, Tokyo 105-8461, Japan
[3]Department of Biochemistry, School of Dentistry, Showa University, Tokyo 142-8555, Japan

Abstract It is now well established that supraphysiological doses of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] stimulate bone resorption. Recent studies have established that osteoblasts/stromal cells express receptor activator of NF-κB ligand (RANKL) in response to several bone-resorbing factors including 1α,25$(OH)_2D_3$ to support osteoclast differentiation from their precursors. Osteoclast precursors which express receptor activator of NF-κB (RANK) recognize RANKL through cell-to-cell interaction with osteoblasts/stromal cells, and differentiate into osteoclasts in the presence of macrophage-colony stimulating factor (M-CSF). Osteoprotegerin (OPG) acts as a decoy receptor for RANKL. We also found that daily oral administration of 1α,25$(OH)_2D_3$ for 14 days to normocalcemic thyroparathyroidectomized (TPTX) rats constantly infused with parathyroid hormone (PTH) inhibited the PTH-induced expression of RANKL and cathepsin K mRNA in bone. The inhibitory effect of 1α,25$(OH)_2D_3$ on the PTH-induced expression of RANKL mRNA occurred only with physiological doses of the vitamin. Supraphysiological doses of 1α,25$(OH)_2D_3$ increased serum Ca and expression of RANKL in vivo in the presence of PTH. These results suggest that the bone-resorbing activity of vitamin D does not occur at physiological dose levels in vivo. A certain range of physiological doses of 1α,25$(OH)_2D_3$ rather suppress the PTH-induced bone resorption in vivo, supporting the concept that 1α,25$(OH)_2D_3$ or its derivatives are useful for the treatment of various metabolic bone diseases such as osteoporosis and secondary hyperparathyroidism. J. Cell. Biochem. 88: 259–266, 2003.

Key words: bone mineralization; bone resorption; osteoclast formation; parathyroid hormone; RANKL

Vitamin D was discovered as an anti-rachitic agent. Vitamin D deficiency leads to impairment of bone mineralization, resulting in rickets in infants and osteomalacia in adults [DeLuca, 1988]. Administration of vitamin D to rachitic animals and humans cures the impaired bone mineralization strikingly. From these results, it was postulated that vitamin D directly stimulates osteoblastic bone formation and mineralization, but even at present, there is no direct evidence for supporting this concept.

It is well recognized that the serum calcium level in healthy humans and animals is tightly regulated from 9 to 10 mg/dl. Intestine, bone, and kidneys are three major organs responsible for this calcium homeostasis. Vitamin D plays a major role in regulating serum calcium homeostasis together with parathyroid hormone (PTH) and calcitonin. Vitamin D acts on all these three organs to increase serum calcium levels, which results in the stimulation of bone mineralization, at least by an indirect mechanism.

VITAMIN D AND BONE MINERALIZATION (BONE FORMATION)

Whether vitamin D directly induces osteoblastic bone mineralization is still a matter of controversy, but the recent findings in vitamin D receptor (VDR) knockout mice have shown an important clue for considering this issue. Homozygotes of VDR knockout mice had no defects in the development and growth before weaning, but their development was strikingly retarded after weaning [Li et al., 1997; Yoshizawa et al., 1997]. At 6 weeks of age, the body weight of the knockout mice was about half of the heterozygotes, and the wild-type mice. Most of the mutant mice died by 15 weeks of age due to hypocalcemia. Bone formation and mineralization

*Correspondence to: Dr. Tatsuo Suda, Division of Pathophysiology, Research Center for Genomic Medicine, Saitama Medical School, 1397 Yamane, Hidaka, Saitama 350-1241, Japan. E-mail: tasuda@saitama-med.ac.jp

Received 2 August 2002; Accepted 3 August 2002
DOI 10.1002/jcb.10331

of the mutant mice were also severely impaired as a typical feature of vitamin D-dependent rickets type II [Li et al., 1997; Yoshizawa et al., 1997].

When VDR knockout mice were maintained for 50 weeks on a rescue diet containing high calcium, they developed normally, and the impaired bone formation and mineralization were recovered completely, although they showed severe alopecia [Amling et al., 1999]. These results clearly indicate that the primary role of vitamin D is to stimulate intestinal absorption of calcium, which in turn promotes bone mineralization indirectly. It is likely that vitamin D does not appear to play an important role in the actual mineralization process of the skeleton, and that the failure to mineralize the skeleton in vitamin D deficiency is due to inadequate levels of calcium and phosphorus in the plasma. Whether vitamin D regulates bone matrix formation and bone quality needs further investigation.

VITAMIN D AND BONE MOBILIZATION (BONE RESORPTION)

It appears paradoxical, but vitamin D functions in the process of calcium mobilization from calcified bone, making calcium available to the extracellular fluid upon demand by the calcium homeostatic system. This important observation was first reported by Carlsson [1952]. When rats were fed a low calcium, vitamin D-deficient diet, the serum calcium level went down from 10 to 5 mg/dl, but it increased to a level of 8 mg/dl 3 days after single administration of 100 U (2.5 μg) of vitamin D_3 to normal rats. In contrast, parathyroidectomized (PTX) rats did not respond at all to vitamin D_3 administration. The rise in serum calcium induced by vitamin D_3 in the presence of PTH was the result of actual mobilization of calcium from bone.

Osteoclasts are the only cells responsible for bone resorption. It is well recognized that osteoclasts are derived from hemopoietic cells of the monocyte–macrophage lineage [Udagawa et al., 1990]. We were interested in the phenomenon that hemopoietic monocytes and macrophages are present in almost all tissues, whereas osteoclasts are present only in bone. This led us to speculate that some local factors or local mechanisms are involved in this tissue-specific localization of osteoclasts in bone. We paid special attention to the role of osteoblasts in osteoclast development, since osteoblasts are present only in bone. The process of osteoclast development consists of several steps: they are proliferation, differentiation, fusion, and activation of osteoclasts. We attempted to develop assay systems to examine each step of osteoclast development in vitro.

In 1988, Takahashi et al. [1988] established an efficient mouse co-culture system to recruit osteoclasts in vitro. Osteoblastic cells were isolated from mouse calvaria, and spleen cells were isolated from splenic tissues and used as osteoclast progenitors. We counted TRAP-positive multinucleated osteoclasts formed in the culture. When osteoblastic cells alone or spleen cells alone were cultured, no osteoclasts were formed even in the presence of $1\alpha,25(OH)_2D_3$. Multinucleated osteoclasts were formed only when spleen cells and osteoblastic cells were co-cultured in the presence of $1\alpha,25(OH)_2D_3$. Cell-to-cell contact between spleen cells and osteoblastic cells appeared important for osteoclast formation, since no osteoclasts were formed when spleen cells and osteoblastic cells were co-cultured but separated by a membrane filter. From these results, we hypothesized that the direct contact of spleen cells and osteoblastic cells is essential for osteoclast differentiation [Suda et al., 1992]. Spleen cells represent osteoclast progenitors, in other words "seeds," and osteoblastic cells represent supporting cells to provide a suitable microenvironment for osteoclast formation in bone, in other words "farm."

MOLECULAR MECHANISM OF BONE RESORPTION

After extensive studies in mouse co-culture system of spleen cells and osteoblastic cells, we proposed a working hypothesis for osteoclastogenesis [Suda et al., 1992] (Fig. 1). The target cells of bone-resorbing factors such as $1\alpha,25(OH)_2D_3$, PTH, and IL-11 were all osteoblastic cells, but not hemopoietic osteoclast precursors. These bone-resorbing factors were classified into three categories in terms of their signal transduction pathways [Suda et al., 1992] (Fig. 1). $1\alpha,25(OH)_2D_3$ induced osteoclast formation via VDR present in the nuclei. PTH, PGE_2, and IL-1 induced osteoclast formation via a protein kinase A system. The third group included IL-6, IL-11, LIF, and Oncostatin M, all of which transduced their signals via gp130.

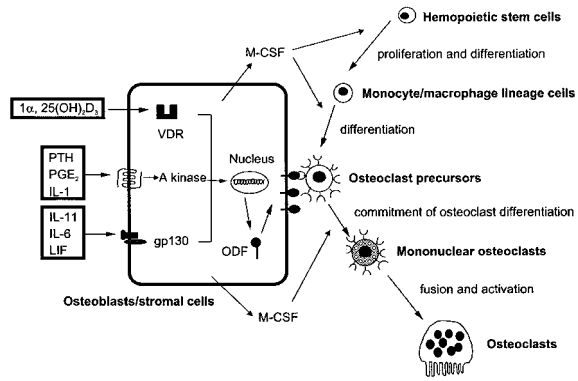


Fig. 1. A hypothetical concept of osteoclast differentiation. Osteotropic factors including 1α,25-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$], parathyroid hormone (PTH), and IL-11 stimulate expression of a membrane-associated factor called osteoclast differentiation factor (ODF) in osteoblasts/stromal cells. Osteoclast progenitors of the monocyte–mactophage lineage recognize ODF through cell-to-cell contact with osteoblasts/stromal cells, then differentiate into osteoclasts in the presence of macrophage-colony stimulating factor (M-CSF).

These three diverse signals appeared to stimulate osteoclast formation independently, since osteoclasts were present both in VDR-knockout mice [Takeda et al., 1999] and in gp130-knockout mice in vivo [Romas et al., 1996]. In other words, there is redundancy in bone-resorbing factors to recruit osteoclasts. We proposed that osteoclast differentiation factor named ODF is commonly induced on the plasma membrane of osteoblastic cells in response to these bone-resorbing factors [Suda et al., 1992, 1995] (Fig. 1). Osteoclast precursors having ODF receptor recognize ODF by cell–cell contact and differentiate into osteoclasts. Macrophage-colony stimulating factor (M-CSF) produced by osteoblastic cells also appeared to play an important role in the proliferation and differentiation of osteoclast progenitors. Thus, it was concluded that osteoblasts are important for osteoclast recruitment in two different ways: one is the production of M-CSF, and the other is the production of a membrane-associated factor like ODF commonly induced by several bone-resorbing factors [Suda et al., 1992, 1995] (Fig. 1).

In 1997, an important clue for the molecular cloning of ODF was made. Two research groups: Amgen in the US [Simonet et al., 1997] and Snow Brand Milk Products in Japan [Tsuda et al., 1997; Yasuda et al., 1998a] independently succeeded in the molecular cloning of a novel inhibitory factor of osteoclastogenesis. The Amgen named this factor "osteoprotegerin (OPG)" due to its protective effect on bone [Simonet et al., 1997]. The Snow Brand named it "osteoclastogenesis inhibitory factor (OCIF)," since they used our co-culture system as an assay system [Tsuda et al., 1997]. The amino acid sequence of OCIF was exactly the same as that of OPG [Simonet et al., 1997; Tsuda et al., 1997].

OPG/OCIF had a signal peptide in the N-terminal region, but did not have a hydrophobic transmembrane domain [Tsuda et al., 1997; Simonet et al., 1997]. This indicates that OPG/OCIF is a secretory protein. OPG/OCIF had four cysteine rich domains designated as CRD I to CRD IV in the N-terminal portion, and two death domain homologous regions designated as DDH1 and DDH2 in the C-terminal portion. The active site of this protein to inhibit osteoclast formation was located in the four cysteine-rich domains [Simonet et al., 1997; Tsuda et al., 1997; Yasuda et al., 1998a].

It is known that all members of the TNF-receptor family possess three to six cysteine-rich domains, in many cases four, in the extracellular portion, which are necessary for the binding of the respective ligands. Fas and TNF receptor p55 possess a death domain in the intracellular region, which is essential for transducing signals for apoptosis.

We hypothesized that ODF could be a common ligand of both ODF receptor and OPG/OCIF. ODF receptor should be located on the plasma membrane of osteoclast progenitors, whereas OPG/OCIF is a secretory protein of the TNF receptor superfamily. In other words, OPG/OCIF present in the blood stream could act as a decoy receptor for ODF to inhibit osteoclast formation. To explore this possibility, we attempted to clone the binding molecule of OPG/OCIF from a cDNA library of bone marrow stromal cells (ST2) treated with $1\alpha,25(OH)_2D_3$.

By screening a cDNA expression library of ST2 cells treated with $1\alpha,25(OH)_2D_3$ and dexamethasone, we finally succeeded in the isolation of a single positive clone, which encoded 316 amino acid residues [Yasuda et al., 1998b]. Hydropathy analysis showed that ODF had no signal sequence, but had an internal hydrophobic domain of 24 amino acid residues, which presumably represents a transmembrane domain. A homology search of the GenBank sequence database revealed that the extracellular C terminal 165 amino acid residues had a significant homology to the members of the TNF

ligand family. This structure was typical of a type II transmembrane protein; its C-terminal sequence exposed outside the cell and the N-terminal sequence intracellularly located [Yasuda et al., 1998b].

Figure 2 summarizes the molecular mechanisms of osteoclast formation and activation [Yasuda et al., 1998b; Suda et al., 1999]. All bone-resorbing factors like $1\alpha,25(OH)_2D_3$, PGE_2, PTH, and IL-11 act on osteoblastic cells to induce a membrane-associated factor called ODF. ODF recognizes osteoclast progenitors having ODF receptor by a mechanism involving cell–cell contact. M-CSF is also an essential factor for osteoclast differentiation, which is produced by osteoblastic cells. Osteoclast progenitors differentiate into osteoclasts by binding to ODF. When OPG/OCIF covers ODF, osteoclast progenitors having ODF receptor are unable to bind ODF, thus osteoclast formation is inhibited.

Figure 3 summarizes the structure and the nomenclature of the ligand, receptor, and decoy receptor of the newly discovered TNF ligand/receptor superfamily members (The ASBMR President's Committee on Nomenclature, 2000). ODF, also called RANK ligand (RANKL) [Anderson et al., 1997], OPG ligand (OPGL) [Lacey et al., 1998], and TNF-related activation-induced cytokine (TRANCE) [Wong et al., 1997], is a new member of the membrane-bound TNF ligand, which consists of 316 amino acid residues and it appears important for the development of T cells and dendritic cells as well as the development of osteoclasts. RANK which has been cloned as a receptor of RANKL consisting of 625 amino acid residues is the transmembrane signaling receptor for ODF as well [Nakagawa et al., 1998]. OPG/OCIF, which consists of 401 amino acid residues is a soluble

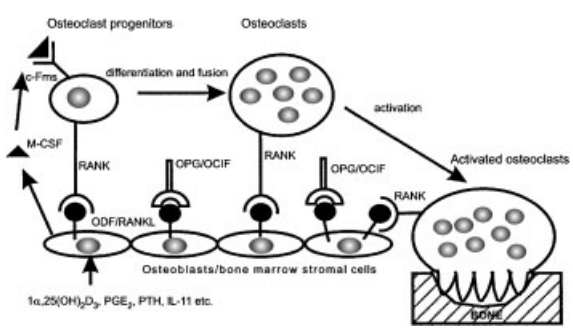


Fig. 2. The molecular mechanism of osteoclast differentiation and activation.

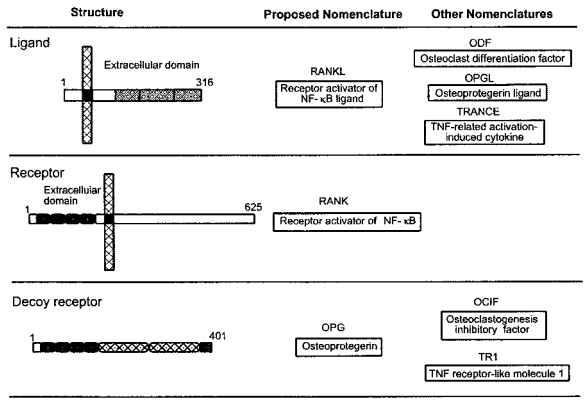


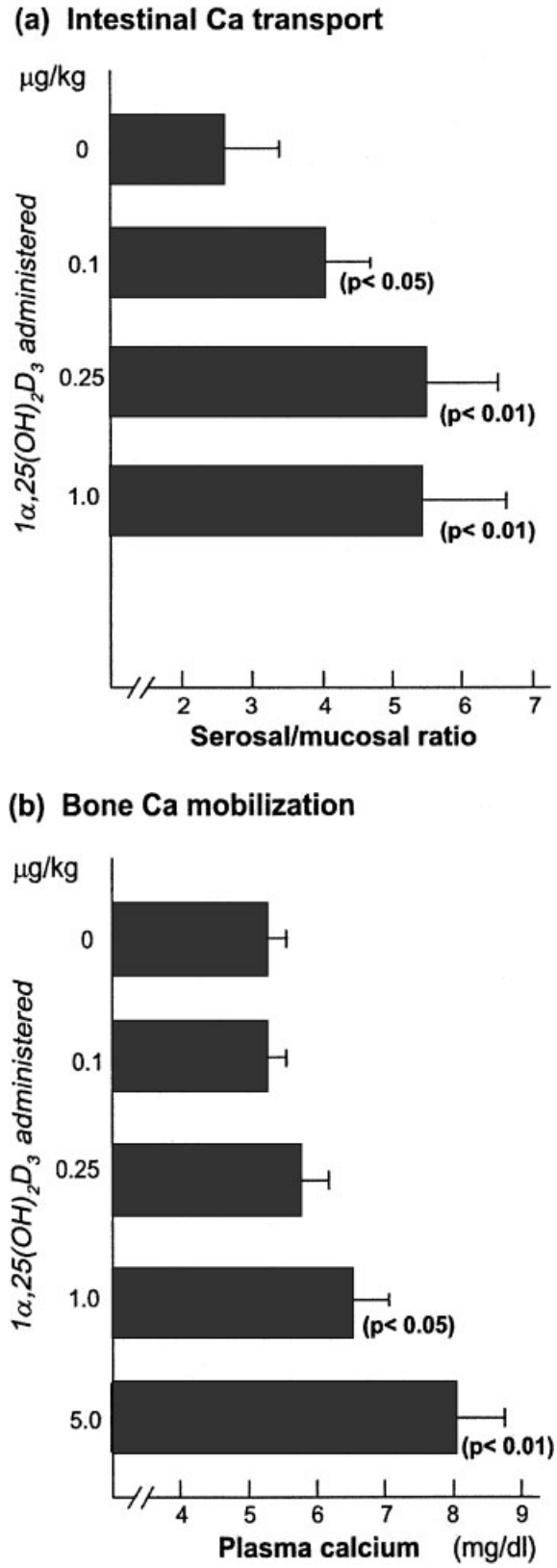
Fig. 3. A schematic representation of the ligand, receptor, and decoy receptor of the newly discovered TNF receptor–ligand family involved in osteoclast formation. Different names for the same ligand/receptors are listed.

receptor for ODF/RANKL, and it functions as a decoy receptor [Yasuda et al., 1998b; Suda et al., 1999].

IN VIVO EFFECTS OF $1\alpha,25(OH)_2D_3$ ON BONE RESORPTION

It is likely that $1\alpha,25(OH)_2D_3$ is a bone-mobilizing hormone, but not a bone-forming hormone at least in vitro. We should emphasize that the bone effects of $1\alpha,25(OH)_2D_3$ depend on its dose levels. Figure 4 shows the differences in the dose levels of $1\alpha,25(OH)_2D_3$ required for inducing intestinal absorption of calcium and bone mineral mobilization activity. In this experiment, graded doses of $1\alpha,25(OH)_2D_3$ were administered to rats fed a low calcium, vitamin D deficient diet. Intestinal absorption of calcium was determined by the routine everted gut sac method, and bone mobilization activity was monitored by measuring serum calcium levels [Suda et al., 1970]. Intestinal absorption of calcium was stimulated by as little as 0.1 μg/kg of $1\alpha,25(OH)_2D_3$, but bone mobilization activity was induced by only 10–50 times more $1\alpha,25(OH)_2D_3$. These results indicate that physiological doses of $1\alpha,25(OH)_2D_3$ do not stimulate bone mobilization. Only supraphysiological or pharamacological doses of $1\alpha,25(OH)_2D_3$ induce bone resorption.

Finally, we examined in vivo relation between vitamin D and PTH in inducing bone resorption. Of several systemic hormones and local factors affecting bone remodeling, vitamin D and PTH may be the most important factors for



regulating bone formation and resorption. In fact, PTH stimulates both bone formation and resorption. Also, supraphysiological or pharmacological doses of $1\alpha,25(OH)_2D_3$ induce bone resorption in vivo, but physiological doses of $1\alpha,25(OH)_2D_3$ do not.

To investigate the dose-dependent effects of vitamin D compounds on bone resorption in a more detail, we examined in vivo effects of $1\alpha,25(OH)_2D_3$ on the expression of RANKL and OPG mRNA in bone of normal and thyroparathyroidectomized (TPTX) rats. When rats were fed a low calcium diet for 3 weeks, serum calcium levels went down from 10.4 to 7.5 mg/dl within 3 weeks. Concomitantly, they showed much higher expression of RANKL and cathepsin K mRNA in bone, compared with rats maintained on an adequate calcium diet. Expression of OPG mRNA in bone did not change appreciably during 1–3 weeks. The rise in the expression of RANKL and cathepsin K mRNA in rats fed a low calcium diet appeared to be due to hyperparathyroidism (Ueno et al., unpublished data).

Thus, rats were TPTX, and similar experiments were conducted in TPTX rats. TPTX decreased the serum calcium level from 9.6 to 5.1 mg/dl. Concomitantly, TPTX greatly reduced the expression of RANKL and cathepsin K mRNA in the cortical bone. The OPG mRNA expression was not changed appreciably by TPTX. Daily oral administration of 0.01 or 0.1 μg/kg of $1\alpha,25(OH)_2D_3$ for 2 weeks had no effect on the expression of all the genes examined, but a supraphysiological dose of 0.5 μg/kg/day of $1\alpha,25(OH)_2D_3$ markedly increased not only serum calcium but also expression of RANKL and cathepsin K mRNA. These results indicate that only supraphysiological or pharmacological doses of $1\alpha,25(OH)_2D_3$ induce bone resorption in vivo (Ueno et al., unpublished data).

To further examine in vivo relation between the role of vitamin D and PTH, we examined the effects of $1\alpha,25(OH)_2D_3$ on the expression of RANKL and cathepsin K mRNA in bone in

Fig. 4. Dose–response effects of $1\alpha,25(OH)_2D_3$ on intestinal Ca transport and bone Ca mobilization activities in vivo. Rats were fed a vitamin D-deficient, low Ca diet for 3 weeks, then received graded doses of $1\alpha,25(OH)_2D_3$. Twenty-four hours later, intestinal Ca transport activity (**a**) and plasma Ca levels (**b**) were measured.

normocalcemic TPTX rats. In these TPTX rats, serum calcium was maintained nearly normocalcemic by constantly infusing PTH at a rate of 50 ng/h with an osmotic mini-pump. These animals had a constant serum PTH level of about 20 pg/ml, which was equivalent to normal rats. They were daily given orally 0.01–0.5 μg/kg of $1\alpha,25(OH)_2D_3$, and gene expression was examined on day 14. Infusion of PTH markedly increased the expression of RANKL and cathepsin K mRNA in bone of TPTX rats. To our surprise, daily administration of 0.01 or 0.1 μg/kg of $1\alpha,25(OH)_2D_3$ suppressed the PTH-induced expression of RANKL and cathepsin K mRNA in bone of TPTX rats. In contrast, a higher dose of 0.5 μg/kg of $1\alpha,25(OH)_2D_3$ did not inhibit the PTH-induced expression of RANKL and cathepsin K mRNA in bone. These results suggest that a certain range of physiological doses of $1\alpha,25(OH)_2D_3$ suppresses RANKL mRNA expression in bone, independent of the suppression of PTH secretion (Ueno et al., unpublished data).

Table I summarizes the dose-dependent effects of the daily oral administration of $1\alpha,25(OH)_2D_3$ for 2 weeks on serum calcium and RANKL mRNA expression in bone. In, in vitro conditions, vitamin D and PTH are capable of inducing bone resorption independently. In contrast, in, in vivo conditions, PTH and vitamin D exhibit a close relationship in inducing bone resorption. PTH is required for inducing bone-resorbing effects of vitamin D, and vice versa. More interestingly, daily administration of 0.01 or 0.1 μg/kg of $1\alpha,25(OH)_2D_3$ is capable of suppressing bone resorption by inhibiting RANKL mRNA expression in normocalcemic TPTX rats constantly infused with PTH.

TABLE I. Dose-dependent effects of 1α,25-Dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$] orally administered on serum Ca and RANKL mRNA expression in bone

	Sham rats	TPTX rats				
PTH continuously infused (50 ng/h)	—	—	+	+	+	+
$1\alpha,25(OH)_2D_3$ orally administered (μg/kg/day)	—	—	—	0.01	0.1	0.5
Serum Ca (mg/dl)	9.6	5.1	10.4	11.1	11.2	14.6
RANKL mRNA expression in bone	—	—	+++	+	+	+++

Ueno, Y. et al, unpublished results

Although $1\alpha,25(OH)_2D_3$ and its synthetic analog, 1α-hydroxyvitamin D_3 [$1\alpha(OH)D_3$], have been widely used for the treatment of many metabolic bone diseases including rickets/osteomalasia, renal osteodystrophy, and osteoporosis [Easel and Riggs, 1998; Reid, 1998], the mechanism of action of $1\alpha,25(OH)_2D_3$ on bone is not clear. Using histomorphometric techniques, Endo et al. [2000] reported that physiological doses of $1\alpha,25(OH)_2D_3$ have a potential role to alleviate hypercalcemia by inhibiting bone resorption in hypercalcemic rats induced by constant PTHrP infusion. Shiraishi et al. [1999] also reported that $1\alpha(OH)D_3$ suppressed the urinary excretion of deoxypyridinoline and exerted bone protective effects even in PTX animals which were rendered normocalcemic by continuous infusion of PTH. From these results, they suggested that $1\alpha,25(OH)_2D_3$ inhibits bone resorption in vivo, independent of the suppression of PTH. The present study further supports the concept that $1\alpha,25(OH)_2D_3$, when administered at physiological doses, is capable of suppressing bone resorption through suppressing PTH-induced RANKL mRNA expression. These in vivo findings contrast with the in vitro results that $1\alpha,25(OH)_2D_3$ stimulates bone resorption in organ cultures [Raisz et al., 1972] and induces the differentiation and activation of osteoclasts in vitro [Suda et al., 1999]. The discrepancy of the action of $1\alpha,25(OH)_2D_3$ on bone resorption and RANKL mRNA induction between in vivo and in vitro appears to be due to the dose levels and the concentration of vitamin D compounds used. Indeed, vitamin D compounds induce hypercalcemia and bone resorption in vivo when administered at supraphysiological or pharmacological doses. The present study confirms this notion.

CONCLUSIONS

Physiological doses of $1\alpha,25(OH)_2D_3$ do not stimulate bone resorption in vivo, but rather inhibit the PTH-induced bone resorption. Suppression of the PTH-induced bone resorption by vitamin D appears to occur by inhibiting the signal transudation pathway of PTH [Miles et al., 2000; Ueno et al., unpublished data]. In order for $1\alpha,25(OH)_2D_3$ to induce bone resorption, supraphysiolgical, or pharmacological doses of the vitamin are required (Fig. 5). Physiological doses of $1\alpha,25(OH)_2D_3$ preferentially stimulate intestinal absorption of calcium without

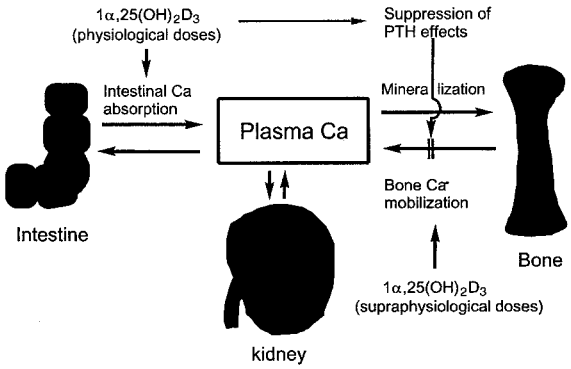


Fig. 5. A hypothetical concept of the dose-dependent effects of $1\alpha,25(OH)_2D_3$ on intestinal Ca transport and bone resorption activities in vivo.

inducing bone resorption, which then stimulates bone mineralization (Fig. 5). These results support the concept that vitamin D compounds are useful for the treatment of various metabolic bone diseases such as osteoporosis and secondary hyperparathyroidism.

REFERENCES

Amling M, Priemel M, Holzmann T, Chapin K, Rueger JM, Baron R, Demay MB. 1999. Rescue of the skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: Formal histomorphometric and biomechanical analyses. Endocrinology 140: 4982–4987.

Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L. 1997. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 390:175–179.

Carlsson A. 1952. Tracer experiments on the effects of vitamin D on the skeletal metabolism of calcium and phosphorus. Acta Physiol Scan 26:212–220.

DeLuca HF. 1988. The vitamin D story: A collaborative effort of basic science and clinical medicine. FASEB J 2:224–236.

Easel R, Riggs BL. 1998. Vitamin D and osteoporosis. In: Feldman D, Glorieux FH, Pike JW, editors. Vitamin D. San Diego: Academic Press. pp 695–711.

Endo K, Katsumata K, Hirata M, Masaki T, Kubodera N, Nakamura T, Ikeda K, Ogata E. 2000. 1,25-Dihydroxyvitamin D_3 as well as its analogue OCT lower blood calcium through inhibition of bone resorption in hypercalcemic rats with continuous parathyroid hormone-related peptide infusion. J Bone Miner Res 15:175–181.

Lacey DL, Timms E, Tan TL, Kelley MJ, Dunstan CR, Burgess T, Eliot R, Colombero A, Elliot G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian Y-X, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. 1998. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93:165–176.

Li YC, Pirro AE, Amling M, Delling G, Baroni R, Bronson R, Demay MB. 1997. Targeted ablation of the vitamin D receptor: An animal model of vitamin D-deficient rickets type II with alopecia. Proc Natl Acad Sci USA 94:9831–9835.

Miles RR, Sluka JP, Santerre RF, Hale LV, Bloem L, Boguslawski G, Thirunavukkarasu K, Hock JM, Onyia JE. 2000. Dynamic regulation of RGS2 in bone: Potential new insights into parathyroid hormone signaling mechanisms. Endocrinology 141:28–36.

Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Yano K, Morinaga T, Higashio K. 1998. RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. Biochem Biophys Res Commun 253:395–400.

Raisz LG, Trummel CL, Holick MF, DeLuca HF. 1972. 1,25-dihydroxycholecalciferol: A potent stimulator of bone resorption in tissue culture. Science 175:768–769.

Reid IR. 1998. Vitamin D and its metabolites in the management of osteoporosis. In: Marcus R, Feldman D, Kelsey J, editors. Osteoporosis. San Diego: Academic Press. pp 1169–1190.

Romas E, Udagawa N, Zhou H, Tamura T, Saito M, Taga T, Hilton DJ, Suda T, Ng KW, Martin TJ. 1996. The role of gp130-mediated signals in osteoclast development: Regulation of interleukin 11 production by osteoblasts and distribution of its receptor in bone marrow cultures. J Exp Med 183:2581–2591.

Shiraishi A, Higashi S, Ohkawa H, Kubodera N, Hirasawa T, Ezawa I, Ikeda K, Ogawa E. 1999. The advantage of Alfacalcidol over vitamin D in the treatment of osteoporosis. Calcif Tissue Int 65:311–316.

Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang M-S, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T. 1997. Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. Cell 89:309–319.

Suda T, DeLuca HF, Tanaka Y. 1970. Biological activity of 25-hydroxyergocalciferol in rats. J Nutr 100:1049–1052.

Suda T, Takahashi N, Martin TJ. 1992. Modulation of osteoclast differentiation. Endocr Rev 13:66–80.

Suda T, Udagawa N, Nakamura I, Miyaura C, Takahashi N. 1995. Modulation of osteoclast differentiation by local factors. Bone 17:87s–91s.

Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. 1999. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 20:345–357.

Takahashi N, Akatsu T, Udagawa N, Sasaki T, Yamaguchi A, Moseley JM, Martin TJ, Suda T. 1988. Osteoblastic cells are involved in osteoclast formation. Endocrinology 123:2600–2602.

Takeda S, Yoshizawa T, Nagai Y, Yamato H, Fukumoto S, Sekine K, Kato S, Matsumoto T, Fijita T. 1999. Stimulation of osteoclast formation by 1,25-dihydroxyvitamin D requires its binding to vitamin D receptor (VDR) in osteoblastic cells: Studies using VDR knockout mice. Endocrinology 140:1005–1008.

Tsuda E, Goto M, Mochizuki S, Yano K, Kobayashi F, Morinaga T, Higashio K. 1997. Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem Biophys Res Commun 234: 137–142.

Udagawa N, Takahashi N, Akatsu T, Tanaka H, Sasaki T, Nishihara T, Koga T, Martin TJ, Suda T. 1990. Origin of osteoclasts: Mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. Proc Natl Acad Sci USA 87:7260–7264.

Wong BR, Rho J, Arron J, Robinson E, Orlinick J, Chao M, Kalachikov S, Cayani E, Bartlett FS, III, Frankel WN, Lee SY, Choi Y. 1997. TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. J Biol Chem 272:25190–25194.

Yasuda H, Shima N, Nakagawa N, Mochizuki S, Yano K, Fujise N, Sato Y, Goto M, Yamaguchi K, Kuriyama M, Kanno T, Murakami A, Tsuda E, Morinaga T, Higashio K. 1998a. Identification of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): A mechanism by which OPG inhibits osteoclatogenesis in vitro. Endocrinology 139:1329–1337.

Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. 1998b. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 95:3597–3602.

Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, Kawakami T, Arioka K, Sato H, Uchiyama Y, Masushige S, Fukamitsu A, Matsumoto T, Kato S. 1997. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia, and growth retardation after weaning. Nat Genet 16:391–396.