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Review Article

Hylonome

Vitamin D analogs and bone

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Abstract

Vitamin D analogs increase intestinal calcium absorption, and have been shown to possess antiresorptive and also bone anabolic properties *in vivo*. Therefore, the pharmacological profile of vitamin D analogs would be well suited for the treatment of osteoporosis. However, the calcemic side effects of this compound class, especially at higher doses, have hampered their wide use in osteoporotic patients. Nevertheless, the clear potential for bone anabolic properties together with oral availability have stimulated the interest in this substance class, and there is an active search for bone selective vitamin D compounds. After an overview of the physiological functions of vitamin D in bone, this article focuses on the effects of acute and chronic administration of pharmacological doses of vitamin D analogs on bone in animal models and humans. Furthermore, the endocrinological, cellular, and microanatomical mechanisms involved in the skeletal actions of vitamin D analogs are discussed. The final section briefly reviews the available data on possible bone selective vitamin D analogs.

Keywords: Calcitriol, Vitamin D, Bone Remodeling, Histomorphometry, Bone Formation, Bone Resorption

Introduction

One of the greatest challenges in the therapy of osteoporosis is to find a cost-effective and safe bone anabolic treatment that would effectively increase bone tissue in an osteopenic skeleton. A number of experimental studies in rats have clearly shown that vitamin D analogs are not only able to prevent estrogen deficiency-induced bone loss by an antiresorptive mechanism[1] but can have pronounced bone anabolic effects at higher dosages[2-5]. Among the other major substance classes that have been shown to possess bone anabolic properties *in vivo*, namely intermittent parathyroid hormone (PTH)[6,7], prostaglandins[8], fibroblast growth factors[9,10], and growth hormone[11,12], vitamin D analogs have the advantage of being orally available. Although fluoride salts are orally available and increase bone mineral density (BMD) in osteoporotic patients, this compound class may lack anti-fracture efficacy[13,14].

In contrast to their clear efficacy in animal studies, vitamin D analogs have not shown uniformly positive results in clinical osteoporosis trials. Although several large studies have suggested that active vitamin D analogs increase BMD in the spine and forearm, and reduce vertebral fractures in osteoporotic patients[15,17], other studies have provided negative results[18]. Therefore, the role of vitamin D metabolites in the treatment of osteoporosis is still controversial. Furthermore, the wide use of vitamin D metabolites in osteoporotic patients, especially at higher doses, has been hampered by the major side effects of this compound class, hypercalcemia and hypercalciuria, due to the excessive stimulation of intestinal calcium absorption. Nevertheless, the clear potential for bone anabolic properties together with oral availability have stimulated the interest in this substance class, and there is an active search for bone selective vitamin D compounds. Women with established postmenopausal osteoporosis often show an increase in bone resorption[19], a decrease in osteoblast team performance[20], and reduced intestinal calcium absorption[21,22]. Therefore, the pharmacological profile of vitamin D analogs would be well suited for the treatment of osteoporosis.

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Physiological functions of the vitamin D hormone in bone

Vitamin D from endogenous, cutaneous synthesis or from dietary sources is ineffective as such and has to be metabolically activated by two hydroxylation steps occurring in the liver and kidney, yielding the vitamin D hormone 1α, 25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] or calcitriol (Fig. 1). Calcitriol is the major biologically active form of vitamin D[23,24], and acts through a nuclear receptor, the vitamin D receptor (VDR). The VDR belongs to the nuclear receptor

superfamily[25], and regulates gene transcription by binding to vitamin D-responsive elements in the promoter region of target genes. Vitamin D deficiency or any block of the subsequent activation steps or of signaling through the VDR results in rickets or osteomalacia.

One of the most important functions of calcitriol in the regulation of calcium homeostasis (Fig. 1) is the stimulation of intestinal absorption of calcium and phosphorus[23]. However, it has not been known until recently whether calcitriol also has an essential function in bone. As osteoblastic cells[26] and early precursor cells for osteoclasts[27] express VDR, physiologically important direct functions of calcitriol in bone would be conceivable. In accordance with this idea, it is well established that calcitriol can modulate osteoblast proliferation and osteoblast production of type I collagen, alkaline phosphatase, and osteocalcin *in vitro*[28]. However, some studies in vitamin D-deficient rats have questioned a direct role of the vitamin D hormone in bone. For example, bone mineralization is normal in vitamin D-deficient rats infused with adequate amounts of calcium and phosphorus[29], and vitamin D-deficient rats can be maintained on a so-called rescue diet for prolonged periods of time without any overt impairment of bone mineralization[30]. The rescue diet is enriched with calcium and phosphorus, and contains a high amount of lactose which stimulates the non-specific intestinal uptake of calcium and phosphorus[31].

The most compelling evidence showing that vitamin D metabolites do not have an essential function in bone comes from studies in VDR knockout mice. Functional inactivation of the VDR by gene targeting in mice creates a condition characterized by severe rickets and alopecia[32,33], similar to hereditary vitamin D-dependent rickets type II, an autosomal recessive disease in humans caused by genetic defects in the VDR gene[34]. Importantly, calcium homeostasis, serum PTH, and all bone abnormalities in VDR-deficient mice can be corrected by feeding the above mentioned rescue diet, starting a few days before weaning[35]. Therefore, the sole function of the vitamin D endocrine system for bone is to provide adequate amounts of calcium and phosphorus for bone mineralization through stimulation of their intestinal absorption (Fig. 1). Calcitriol does not have physiologically important direct functions in bone. Furthermore, these experiments have conclusively shown that calcitriol is not directly involved in the control of PTH secretion in the parathyroid glands under physiological circumstances. Thus, all the skeletal effects of vitamin D deficiency are mediated indirectly through decreased intestinal mineral absorption and the accompanying secondary hyperparathyroidism.

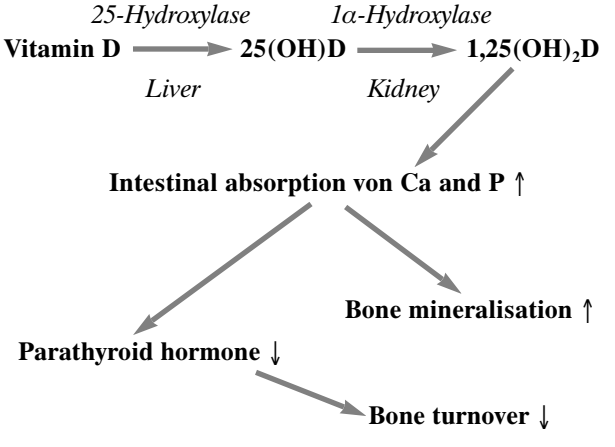

Figure 1. Physiological function of the vitamin D hormone for calcium homeostasis and in bone. Vitamin D from endogenous synthesis in the skin or from dietary sources is activated by two hydroxylation steps occurring in the liver and kidney, to yield the vitamin D hormone 1α,25-dihydroxyvitamin D_3 or calcitriol. The sole physiological function of calcitriol for calcium homeostasis is the stimulation of the intestinal absorption of calcium and phosphorus. Both minerals are needed for bone mineralization. Through the calcium-sensing receptor, parathyroid hormone (PTH) secretion in the parathyroid glands is tightly regulated by extracellular calcium. Among other actions, PTH is the major determinant of bone turnover under physiological circumstances. Therefore, a rise in extracellular calcium suppresses PTH secretion, which in turn down-regulates bone turnover.

An important inactivating pathway for vitamin D metabolites is 24-hydroxylation occurring in the kidney and in most target tissues of calcitriol[23]. Over many years, there has been considerable controversy as to whether 24-hydroxylated vitamin D metabolites such as 24R,25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$] or 1α,24R,25-trihydroxyvitamin D_3 [1,24,25-$(OH)_3D_3$] may have specific functions in bone. Experiments using 24,24-difluoro-25-hydroxyvitamin D_3, a synthetic compound that cannot be hydroxylated in the 24 position, have provided evidence that 24-hydroxylation is neither necessary for healing of rachitic bone lesions nor for normal development, modeling, and mineralization of bone in vitamin D-deficient rats[36-39]. On the other hand, a number of studies have suggested a specific role for 24,25$(OH)_2D_3$ in bone mineralization, endochondral bone formation, proliferation and differentiation of growth plate chondrocytes, and matrix calcification in growth plate cartilage (reviewed in[40]). However, recent investigations in mice with a targeted inactivating mutation of the 24-hydroxylase gene have conclusively demonstrated that 24-hydroxylated vitamin D metabolites are not necessary for normal bone development[41]. These 24-hydroxylase knockout mice show impaired intramembranous bone mineralization due to defective catabolism of calcitriol with subsequent build-up of very high serum concentrations of calcitriol. However, the bone phenotype of the 24-hydroxylase mutant mice is rescued when they are crossed with VDR knockout mice showing that the elevated calcitriol levels, acting through the VDR, are responsible for the observed impairment of bone mineralization and accumulation of osteoid[41].

Skeletal effects of acute, pharmacological administration of vitamin D analogs

Despite the well-established lack of a direct physiological function of vitamin D metabolites in bone, pharmacological

administration of vitamin D analogs induces profound changes in bone metabolism that are very likely mediated through a local action on bone cells. Because acute and chronic administration of vitamin D analogs produce opposite effects *in vivo*, the two treatment modalities are described separately here.

Calcitriol is a potent stimulator of bone resorption *in vitro*[27], and a single intravenous injection of calcitriol at the very high dose of 1 μg/kg in vitamin D-deficient, thyroparathyroidectomized rats results in an augmentation of osteoclast activity and recruitment that occurs within 6 - 48 hours[42]. In this model, ultrastructural signs of osteoclast activation are seen as early as 6 hours after acute calcitriol administration, while an increase in osteoclast number can be shown after 2 days[42]. Similarly, daily intraperitoneal doses of 0.8 μg/kg calcitriol given to intact rats increase osteoclast number after 1 day[43]. Based mainly on *in vitro* findings, the initial increase in osteoclast number and activity after high dose calcitriol is thought to be caused by enhanced monocytic differentiation of immature hematopoietic cells and subsequent commitment of monocytic cells into preosteoclasts, by increased fusion of osteoclast precursors, and by augmented cell activity of mature osteoclasts (reviewed in[27,44]). Mature osteoclasts do not express VDR, and the latter effect is mediated through cells of the osteoblastic lineage (reviewed in[27,45]). Calcitriol alters the expression of receptor activator of NF-kB ligand (RANKL) and of osteoprotegerin (OPG) in osteoblasts (reviewed in[46]), thereby regulating osteoclast differentiation and the activity of mature osteoclasts. Both factors are essential molecules for the control of osteoclast formation and function. The enhanced differentiation of hematopoietic precursors into the monocytic lineage induced by calcitriol may involve an additional, direct action on promyelocytes which contain VDR[47,48]. The increase in bone resorption seen after acute administration of high dose calcitriol is undoubtedly caused by a direct effect on bone because it occurs in the absence of PTH in thyroparathyroidectomized rats[42], and also in vitamin D-deficient rats kept on a zero-calcium[49] or low-calcium diet (0.02% Ca)[50].

However, surprisingly, when the calcitriol treatment is further continued, the initial stimulation of bone resorption turns into a suppression of bone resorption. In a histomorphometric study in rats, daily intraperitoneal injections of 0.8 μg/kg calcitriol increased osteoclast numbers in tibial cancellous bone on day 1, but diminished osteoclast numbers on days 6, 8, and 10 of the study[43]. A transient reduction in osteoclast number on day 7 has also been reported when systemic calcitriol treatment with daily doses of 0.2 μg/kg was stopped after 3 days[51]. Because the suppression of osteoclast number after a 10-day calcitriol treatment does not occur in rats on a low-calcium diet[52], it is likely that this effect is caused by PTH suppression. In parallel to the augmentation of bone resorption, intestinal calcium absorption is increased within 1-2 days under treatment with vitamin D analogs in animals on a normal diet[53]. Both the increased intestinal absorption of calcium and the increased release of calcium from bone through enhanced resorption result in a rise in blood calcium and a subsequent suppression of PTH secretion after a few days of treatment[51]. In addition, PTH secretion may also be suppressed by a direct inhibitory effect of vitamin D analogs on the parathyroid glands[54-57]. Because PTH is the most important hormonal regulator of osteoclast activity and recruitment under physiological circumstances, the PTH suppression induced by external administration of calcitriol probably overrides the direct stimulating effects of calcitriol on bone resorption, thereby depressing osteoclast number and activity after a few days of acute calcitriol administration. The excess calcium in the extra-cellular compartment of calcitriol-treated rats is excreted via the urine[51], favored by the low circulating PTH. A reduction in serum PTH down-regulates renal tubular reabsorption of calcium.

Apart from the effects on bone resorption, vitamin D analogs also have profound influences on bone formation. A single oral dose of 0.1 μg/kg calcitriol up-regulates tibial osteocalcin mRNA levels, and increases serum osteocalcin within 6 hours in rats[58]. Daily parenteral doses of 0.8 μg/kg calcitriol profoundly increase osteoblastic matrix formation with subsequent accumulation of osteoid in rat cancellous bone within days[43]. The electron microscopic study by Boyce et al.[59] reported ultrastructural signs of osteoblast hypertrophy and increased matrix production, 4 days after start of daily intraperitoneal administration of 0.8 μg/kg calcitriol to rats. In a similar fashion, the number of osteoblast precursor cells in bone marrow was shown to be increased after a 3-day treatment with 0.2 μg/kg calcitriol[51]. Therefore, in parallel to the stimulation of bone resorption, osteoblast activity and recruitment is augmented by calcitriol. Because the effects of calcitriol on osteoblastic matrix formation in these *in vivo* experiments are always accompanied by hypercalcemia it is unclear whether the stimulating effects of calcitriol on bone formation are direct or indirect[51]. In support of an indirect component of action, the rat study by Boyce and Weisbrode[52] has shown that bone matrix formation induced by a 10-day calcitriol therapy is modulated by dietary calcium. In contrast, the very early effects, e.g., up-regulation of osteocalcin mRNA within a few hours after a single dose of calcitriol[58], suggest that there may also be a direct action of vitamin D analogs on bone-forming cells.

In all of these short-term rat studies, high or very high parenteral or oral doses of calcitriol (above 0.1-0.2 μg/kg per day) have been used. Therefore, it is not known whether lower doses also induce an initial stimulation of osteoclastic bone resorption. Clinical studies using biochemical markers of bone resorption such as urinary hydroxyproline and serum C-terminal telopeptide of type I collagen (ICTP) suggested that short-term treatment with lower oral doses of calcitriol (1-4 μg per day, i.e., about 0.015 – 0.06 μg/kg/day) does not cause such an initial resorptive phase in humans[60-62]. Therefore, acute administration of calcitriol in a dose range that can be given to humans without the risk of serious side effects may not cause the initial increase in bone resorption seen after treatment with high dose calcitriol in animal studies. However, similarly to the findings in rats, all of the human

studies demonstrated a stimulation of bone formation after calcitriol therapy. Geusens et al.[60] reported a rise in serum osteocalcin after a 4-day treatment of postmenopausal women with 4 μg calcitriol/day. Gram et al.[62] administered calcitriol in a dose range of 1 to 2 μg/day to healthy male volunteers for 7 days and could show a dose-dependent increase in serum levels of osteocalcin and procollagen type I C-terminal propeptide (PICP) within 24 hours after start of the treatment.

Taken together, acute administration of high dose calcitriol to rats causes a transient increase in bone resorption that lasts for about 2-3 days. Lower doses may not induce a pronounced initial resorptive phase. Later on, bone resorption is suppressed under continuing therapy. In parallel to the effects on bone resorption, bone formation is stimulated, and osteoblast recruitment and osteoblastic matrix production is increased after about 4 days of high dose calcitriol therapy.

Skeletal effects of chronic administration of vitamin D analogs

It is well established that chronic administration of active vitamin D metabolites to rats under the conditions of a sufficient dietary calcium intake results in a dose-dependent suppression of bone resorption[1-5,52,63-70]. The same is true for humans[71,72]. Even toxic doses that induce weight loss in rats still suppress bone resorption as assessed by osteoclast numbers in vertebral cancellous bone and as measured by urinary collagen crosslinks excretion at the whole body level[5]. As outlined above, the main mechanism for the antiresorptive effect of chronic vitamin D analogs *in vivo* appears to be PTH suppression (Fig. 2). This notion is corroborated by the finding that the suppressive effect of calcitriol on osteoclast number in ovariectomized (OVX) rats is modulated by dietary calcium[4], and that calcitriol treatment fails to suppress osteoclast number in intact rats on a low-calcium diet[52]. However, experiments in parathyroidectomized rats infused over 2 weeks with human PTH-related peptide(1-34) and simultaneously orally treated with calcitriol or 22-oxacalcitriol showed that vitamin D analogs are able to dose-dependently reduce serum calcium in this model of humoral hypercalcemia of malignancy, suggesting a direct antiresorptive effect on bone[73]. Nevertheless, the available evidence suggests that such a direct antiresorptive pathway is of minor importance for chronic treatment with vitamin D analogs under normal circumstances.

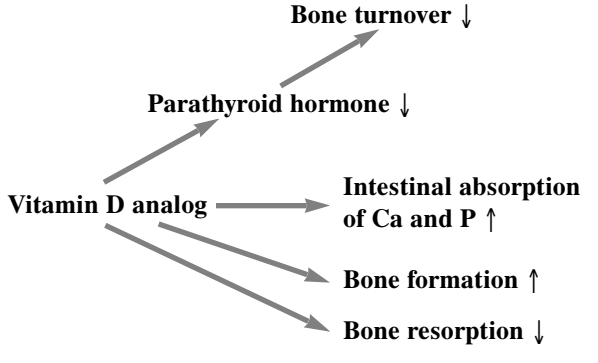

Figure 2. Effects of pharmacological treatment with vitamin D analogs on bone. External administration of an active vitamin D analog dose-dependently lowers PTH secretion indirectly via stimulation of intestinal calcium absorption and a subsequent rise in serum calcium, and by a direct inhibitory activity on the parathyroid gland at the transcriptional level. PTH suppression down-regulates bone turnover, i.e., bone resorption and bone formation. At higher dosages, bone formation is stimulated through a direct action on bone-forming cells or their precursors. This effect overrides the suppression of bone formation by diminished PTH serum levels. Recent *in vivo* data have suggested that chronic treatment with vitamin D analogs also may have a direct suppressive effect on bone resorption mediated through cells of the osteoblastic lineage. At present, the relevance of this latter pathway is unclear.

Due to their antiresorptive effect, low oral doses of vitamin D analogs are able to prevent cancellous and cortical bone loss induced by estrogen deficiency[1,3,5,64-70,74] or immobilization[63,75-77] in rats, and correct cancellous bone loss in ovariohysterectomized dogs[78]. Additionally, when higher doses are given, cancellous bone mass in nongrowing or slowly growing bone sites increases above the level seen in baseline controls or in sham-operated control animals[3,4,64,69,79]. For example, therapeutic administration of calcitriol to 9-month-old, osteopenic OVX Fischer rats increased lumbar vertebral cancellous bone mass relative to vehicle-treated OVX controls by 39% in a three-month trial[4]. Longitudinal bone growth is undetectable in lumbar vertebrae of female Fischer rats at this age[80]. It is known that purely antiresorptive drugs such as bisphosphonates do not increase cancellous bone volume in nongrowing bone sites[81]. Therefore, the antiresorptive activity cannot account for the bone anabolic effect induced by vitamin D analogs in nongrowing bone sites. Rather, such an effect must involve a net increase of bone formation over bone resorption.

In addition, recent studies in OVX rats have shown that high doses of vitamin D analogs increase the total cross-sectional area of the tibial shaft[5]. Therefore, vitamin D analogs are not only anabolic for cancellous but also for cortical bone through stimulation of periosteal bone apposition. A positive effect of vitamin D metabolites on cortical bone in rats has also been suggested by earlier studies[74,82]. The increase in cancellous and cortical bone mass induced by calcitriol in animal studies is paralleled by similar increases in biomechanical bone strength[70].

Previous investigations have shown that chronic administration of calcitriol to rats at dosages higher than about 0.1 to 0.2 μg/kg can result in impaired bone mineralization[2,83]. Similarly, very high levels of endogenous calcitriol in mice with a defective vitamin D catabolism induce impaired bone mineralization[41]. However, when lower dosages of calcitriol are given, bone mineralization is usually not adversely affected. Under chronic treatment with low or moderately high doses of vitamin D analogs, the increase in osteoid width is counteracted by a rise in mineral apposition rate, so that osteoid maturation rate is unchanged or may even be reduced[5,70].

A very important question is whether the bone anabolic

actions of vitamin D analogs are direct or indirect. The development of bone specific compounds with reduced effects on intestinal calcium absorption is only possible on the basis of a direct action of vitamin D analogs on bone. In the absence of predictable *in vitro* systems mimicking the bone anabolic action of vitamin D analogs *in vivo*, this question is not trivial to address. Experiments with short-term treatment (10 days) of rats with high dose calcitriol have shown that the calcitriol-induced increase in osteoblastic matrix synthesis depends on an adequate supply of dietary calcium, and does not occur in rats on a low calcium diet (0.05% calcium, 0.3% phosphorus)[52]. A possible confounding factor in these studies is that adaptation of, especially growing, rats to diets with a very low calcium content induces profound changes in bone metabolism that may override any pharmacological effect of calcitriol on bone.

Indeed, a 3-month study in OVX rats maintained under steady state conditions on a diet with a reduced calcium content (0.25% calcium, 0.6% phosphorus) showed that the anabolic action of two different doses of calcitriol on vertebral cancellous bone was not modulated by a large dietary calcium supplement, and there was no interaction between calcitriol and calcium[4]. The opposite would be expected if the anabolic actions of calcitriol on bone were mediated indirectly through alterations in calcium homeostasis. Therefore, these results support the notion that the increase in cancellous bone mass induced by chronic treatment with vitamin D analogs is based on a direct, pharmacological effect on bone. Another line of evidence strengthens this idea. Transgenic mice overexpressing human VDR in mature osteoblasts under the control of an osteocalcin promoter have increased trabecular thickness, increased cancellous bone volume, decreased osteoclast surface, and increased cortical bone[84]. It is likely that overexpression of VDR in mature osteoblasts makes these cells more responsive to the actions of endogenous calcitriol. Interestingly, the observed phenotype in the VDR transgenic mice closely resembles the one obtained by pharmacological treatment of rats with higher doses of vitamin D analogs[3,5,51].

In accordance with a direct anabolic action of vitamin D analogs on bone, parameters of bone formation typically show a biphasic response to increasing doses of a vitamin D compound. Low doses suppress bone formation in OVX rats, whereas high doses increase bone formation rate and augment histomorphometric parameters of osteoblast team performance such as mineral apposition rate and wall width of completed remodeling units[3,5,20]. The suppressive effect of low doses on bone formation is probably due to PTH suppression resulting in a down-regulation of bone turnover, i.e., down-regulation of both bone resorption and of bone formation (Fig. 2). Higher doses, on the other hand, stimulate bone-forming cells in the presence of further diminished serum PTH through a direct pathway (Fig. 2).

So far, the available data suggest that the skeletal response to vitamin D analogs is similar in rats and humans, and rats may be predictive of the human response to a given compound. However, it is important to note that the effects of chronic treatment with vitamin D analogs on bone may greatly differ between different animal species. In rabbits, for example, calcitriol was shown to exaggerate disuse osteoporosis and glucocorticoid-induced osteoporosis, and to impair fracture healing[85]. Other studies in rabbits, however, have demonstrated positive effects of chronic administration of vitamin D analogs on bone mass[86], and on callus formation during distraction osteogenesis[87]. Although, as mentioned above, overexpression of VDR in osteoblasts has positive skeletal effects in mice, chronic administration of vitamin D analogs at high doses appears to induce bone loss in this species[88,89]. In contrast, vitamin D analogs increase bone mass in rats and humans. Therefore, similar to the situation with some other steroid hormones, mice may not be a good model for the skeletal actions of vitamin D analogs in humans.

Cellular and microanatomical mechanisms of the bone anabolic action of vitamin D metabolites

A very typical feature of the bone anabolic effects of vitamin D analogs in cancellous bone is an increase in trabecular width[3,5,68,70]. There are two mechanisms that can account for such a thickening of existing bone structural elements. One possibility is the induction of mini-modeling drifts on quiescent bone surfaces, the other is the generation of a positive remodeling balance. A positive remodeling balance, i.e., the quantum of bone formed during the formation phase of the remodeling cycle exceeds the quantum of bone previously resorbed by osteoclasts in the same site, results in increasing thickness of bone trabeculae with every completed remodeling cycle. Higher doses of vitamin D metabolites increase the wall width of completed remodeling units[3,5,70,90]. Morphological evidence (Fig. 3A) as well as the very strong relationship between trabecular width and wall width in rats treated with vitamin D analogs[3] suggest that the main microanatomical mechanism by which moderately high doses of these compounds increase cancellous bone mass is probably the induction of a positive remodeling balance.

However, high to very high doses of vitamin D compounds also induce typical "boutons" of newly formed bone on smooth trabecular surfaces (Fig. 3B). These structures are indicative of locally confined mini-modeling drifts. During mini-modeling, either bone formation or bone resorption follows activation[91,92]. Mini-modeling normally functions to realign or to strengthen bone spicules[93]. Under stimulation with very high doses of vitamin D analogs, localized formation drifts originating from resting bone surfaces can reconnect adjacent bone trabeculae (Fig. 3C).

It has been suggested that the wall width of completed remodeling units is a function of the performance of individual osteoblast teams[20]. How can the positive effect of vitamin D analogs on osteoblast team performance be explained? There is no conclusive answer to this question yet. However, vitamin D analogs increase the number of osteoblast precursor cells

in bone marrow[51], and may, therefore, increase the number of cells within a given osteoblast team. This increased number of bone-forming cells may then be translated into augmented performance of the osteoblast team during the formation phase of the remodeling cycle[20]. Alternatively, vitamin D analogs may stimulate the activity of individual osteoblasts already engaged in bone formation[94]. It is possible that both mechanisms contribute to the bone anabolic action of vitamin D analogs[51].

At present, it is unclear whether such a "cell mechanistic" view can explain the bone structural phenomena induced by bone anabolic agents such as vitamin D metabolites. The structure of bone is highly controlled by its biomechanical environment[92]. It has been proposed that bone anabolic agents may sensitize osteocytes and osteoblasts to mechanical stimuli so that they "over-react" to their normal biomechanical environment, falsely increasing the strength of the structural elements[95-97]. It is possible that such a process may result in the observed effects under therapy with high doses of vitamin D analogs, i.e., overfilling of resorption cavities, induction of mini-modeling drifts, and increased periosteal bone apposition.

Furthermore, it has been proposed that bone anabolic agents may act through increasing muscle strength[97,98]. However, it is unlikely that this scenario applies to vitamin D analogs. Even toxic doses of vitamin D metabolites resulting in severe hypercalcemia and weight loss still profoundly increase cancellous and cortical bone mass in rats[5]. Further, hypercalcemia is generally associated with muscle weakness due to its membrane-stabilizing effect[23]. Therefore, although it cannot be totally ruled out, it is unlikely that the bone anabolic actions of vitamin D analogs are mediated through increased muscle strength.

Are there bone specific vitamin D analogs?

The most interesting aspect of vitamin D analogs for the treatment of osteoporosis is their clear bone anabolic efficacy. However, in order to exploit the bone anabolic potential of vitamin D analogs, high doses of the compounds have to be given. Due to the calcemic side effects, such a treatment is not feasible with compounds that are equally calcemic compared with calcitriol. Because, as outlined above, the bone anabolic effects of vitamin D analogs have a direct component, the design of bone selective compounds appears to be possible. Are there existing examples for bone selective vitamin D analogs?

One possible approach for the development of bone specific vitamin D analogs would be to make use of tissue selective local activation of a prodrug[24]. Indeed, a study in 3-month-old OVX rats[67] had suggested that the synthetic prodrug 1α-hydroxyvitamin D_2 (1α(OH)D_2) combines at least equal bone-preserving activity with distinctly reduced calcemic effects relative to 1α-hydroxyvitamin D_3 (1α(OH)D_3). 1α(OH)D_2 and 1α(OH)D_3 are inactive as such and have to be metabolically activated, a process that occurs mainly in the liver[99,100]. It has been shown that primary mouse osteoblasts contain vitamin D_3 25-hydroxylase mRNA, and can metabolize 1α(OH)D_3 into 1,25$(OH)_2D_3$ *in vitro*[101]. Thus, 1α(OH)D_3 and 1α(OH)D_2 may partially be activated locally in osteoblasts, and it would be conceivable that the apparent tissue selectivity for 1α(OH)D_2 could be explained by tissue selective differences in activation. However, a subsequent dose response study in 6-month-old OVX rats confirmed the lower toxicity of 1α(OH)D_2 compared with 1α(OH)D_3 but revealed no evidence for selective metabolic activation of 1α(OH)D_2 in bone[5]. The reason for the reduced toxicity of 1α(OH)D_2 is still unclear.

ED-71 is an A ring modified synthetic analog of calcitriol with increased affinity for the vitamin D binding protein, and, thus, a prolonged serum half life[102]. This compound has been developed for the treatment of osteoporosis. However, ED-71 stimulates intestinal calcium absorption and *in vivo* bone calcium mobilization in a similar fashion compared with calcitriol[102]. Therefore, although this compound may have advantages over calcitriol or 1α(OH)D_3 in the therapy of

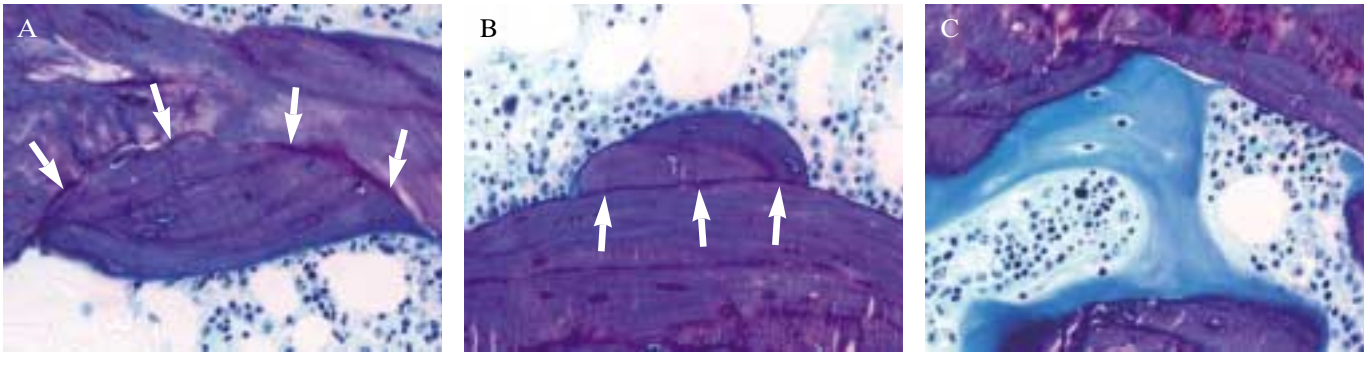

Figure 3. Microanatomical mechanisms of the bone anabolic effect of vitamin D analogs. A. Chronic treatment with moderate to high doses of vitamin D analogs has positive effects on remodeling balance, resulting in overfilling of resorption cavities and a subsequent increase in trabecular width. The reversal line is marked with arrows. B. High doses of vitamin D analogs induce the formation of typical "boutons" of newly formed matrix on resting bone surfaces indicated by the smooth cement line (arrows). C. Under stimulation with very high doses of vitamin D analogs adjacent bone spicules can become reconnected by excessive osteoblastic matrix formation. Note the accumulation of unmineralized osteoid (light blue). Rat vertebral cancellous bone. Animals were treated for 3 months with calcitriol, using daily oral doses of 0.1 μg/kg (A and B) or 0.2 μg/kg (C). Toluidine blue surface stain for demonstration of cement lines. Original magnification x400.

bone diseases[66,90], it does not appear to be bone selective. The same appears to be true for other analogs developed for osteoporosis treatment such as 26,27-F_6-1α,25$(OH)_2D_3$[76] and 24-epi-1α,25$(OH)_2D_2$[103], or the natural vitamin D metabolite 1α,24,25$(OH)_3D_3$[3].

Thus, so far no convincing published evidence has been provided for a bone specific vitamin D analog. Hopefully, however, bone selective compounds will be synthesized and characterized in the near future. Especially the absence of reliable *in vitro* assays for the bone anabolic actions of vitamin D analogs has hampered screening for bone selective compounds in the past. Therefore, it is an important goal for skeletal research to improve our understanding of the molecular pathways and the target cells involved in the bone anabolic and also the antiresorptive effects of this compound class.

Conclusions

Although vitamin D metabolites lack a direct physiological function in bone, pharmacological administration of vitamin D analogs induces profound changes in bone metabolism that are partially mediated through a local action on bone cells. Externally administered vitamin D analogs suppress bone resorption mainly by an indirect effect involving PTH suppression. The most interesting aspect of the skeletal effects of vitamin D analogs is their clear bone anabolic property at higher dosages. At least partially, the anabolic action of vitamin D analogs is based on a direct, pharmacological effect on bone. However, due to the calcemic side effects, the exploitation of the bone anabolic effects of vitamin D analogs in clinical medicine will require bone selective vitamin D analogs. So far, there is no convincing evidence for such a bone selective drug. For the development of bone specific vitamin D analogs it would be of great importance to gain further insight into the molecular pathways and the target cells involved in the bone anabolic and antiresorptive effects of this compound class.

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