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Differential effects of Vitamin D analogs on bone formation and resorption

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Abstract

Deficiency in Vitamin D and its metabolites leads to a failure in bone formation primarily caused by dysfunctional mineralization, suggesting that Vitamin D analogs might stimulate osteoblastic bone formation and mineralization. In this study, we compare the effect of selected Vitamin D analogs and active metabolite, 1α,25-dihydroxyvitamin D_3, 19-nor-1α, 25-dihydroxyvitamin D_2, and 1α-hydroxyvitamin D_2 or 1α,25-dihydroxyvitamin D_2 on bone formation and resorption.

In a mouse calvariae bone primary organ culture system, all Vitamin D analogs and metabolite tested-stimulated collagen synthesis in a dose-dependent manner and 19-nor-1α, 25-dihydroxyvitamin D_2 was the most efficacious among three. 19-nor-1α, 25-dihydroxyvitamin D_2 and 1α,25-dihydroxyvitamin D_2 showed similar potencies and 1α,25-dihydroxyvitamin D_3 was less potent than others. Osteocalcin was also up-regulated in a dose-dependent manner, suggesting that the three Vitamin D analogs have the equal potencies on bone formation. 25-Hydroxyvitamin D-24-hydroxylase expression was induced in a dose-dependent manner and 19-nor-1α, 25-dihydroxyvitamin D_2 was less potent than other two compounds.

In a mouse calvariae organ culture, all induced a net calcium release from calvariae in a dose-dependent manner, but the potency is in the order of 1α,25-dihydroxyvitamin $D_2 \cong$ 1α,25-dihydroxyvitamin $D_3 >$ 19-nor-1α, 25-dihydroxyvitamin D_2. In a Vitamin D/calcium-restricted rat model, all caused an elevation in serum calcium in a dose-dependent manner. There is no significant difference between 1α,25-dihydroxyvitamin D_3 and 1α-hydroxyvitamin D_2 in potencies, but 19-nor-1α, 25-dihydroxyvitamin D_2 is at least 10-fold less potent than the other two compounds.

Our results suggest that Vitamin D analogs have direct effects on bone resorption and formation, and 19-nor-1α, 25-dihydroxyvitamin D_2 may be more effective than 1α,25-dihydroxyvitamin D_3 and 1α-hydroxyvitamin D_2 on stimulating anabolic bone formation.

Keywords: Vitamin D analogs; VDR; Calvariae; Bone resorption; Bone formation

1. Introduction

Vitamin D_2 or D_3 is modified by 25-hydroxylase in the liver and 25-hydroxyvitamin D 1α-hydroxylase in the kidney to form the active metabolite, 1,25-dihydroxyvitamin D_2 or D_3 (calcitriol), which is then metabolized by 25-hydroxyvitamin D-24-hydroxylase (24-OHase, CYP24A1) [1,2]. The binding of 1,25-dihydroxyvitamin D_2 or D_3 or their analogs to Vitamin D receptor (VDR), a nuclear receptor, activates VDR to interact with retinoid X receptor (RXR) and forms the VDR/RXR/cofactor complex, which binds to Vitamin D response elements in the promoter region of target genes to regulate gene transcription [3].

The kidney is the major site of 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1), which is responsible for the activation of 25-hydroxyvitamin D. As renal disease progresses, inadequate renal phosphate clearance and reduced calcium reabsorption to maintain ionized calcium levels within an optimal range lead to chronic over-stimulation of

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parathyroid hormone (PTH) synthesis. Consequently, secondary hyperparathyroidism is a frequent complication in chronic renal disease characterized by parathyroid hyperplasia and enhanced synthesis and secretion of PTH. In addition, patients with chronic renal disease often experience renal osteodystrophy characterized by high, low or mixed turnover bone diseases because 1,25-dihydroxyvitamin D plays a key role in regulating the deposition of calcium and phosphorus into the bone [2]. Vitamin D analogs and active metabolite, such as 1α,25-dihydroxyvitamin D_3, the endogenous active metabolite, 19-nor-1α, 25-dihydroxyvitamin D_2, an analog of 1α,25-dihydroxyvitamin D_2, and 1α-hydroxyvitamin D_2, which is activated in vivo into 1α,25-dihydroxyvitamin D_2, are commonly used to manage secondary hyperparathyroidism and renal osteodystrophy associated with chronic kidney disease [1,4].

Targeted ablation of the VDR gene [5] in mice results in not only rickets but also osteomalacia. Normalization of mineral ion homeostasis by a rescue diet high in lactose, calcium and phosphorus greatly improves the serum PTH concentration and corrects the osteomalacia defect in these animals. However, upon closer examination, it was found that, although the length of the femur was normalized, the rescue diet failed to normalize bone mineral density. Furthermore, the bone volume of the mice was reduced due to decreased bone formation. On the other hand, both the length and the density of the femur in the CYP27B1 knockout mice treated with 1,25-dihydroxyvitamin D_3 are not significantly different from the wild type [6]. These observations suggest that VDR activators play a direct role in controlling bone formation/resorption, and raise the question of whether it is sufficient to control mineral ion homeostasis in the chronic renal disease patients without Vitamin D analog therapy.

Finch et al. demonstrated that 19-nor-1,25-dihydroxyvitamin D_2 is 10 times less effective in mobilizing calcium and phosphorus from the skeleton compared with 1,25-dihydroxyvitamin D_3 [7], but has the same potency in osteocalcin levels, alkaline phosphatase activity, and controlling osteoblastic growth [8,9]. On the other hand, 1alpha-hydroxyvitamin D_2 prevent cortical bone loss in OVX rats and have anabolic effects on cortical bone, but induce intracortical remodeling as an untoward side effect at high doses [10]. Although the relationship between Vitamin D and the bone have been the focus of research efforts for some time, inconsistency exists in data regarding its direct role on bone [11–13]. The issue is compounded in the in vivo studies in which the effects of Vitamin D on calcium and phosphate homeostasis make it difficult to delineate whether Vitamin D is directly or indirectly involved in bone metabolism. In addition, limited studies have been done to compare the direct effect of various Vitamin D analogs on bone resorption/formation.

In this study, we examined the effects of selected Vitamin D analogs and active metabolite, 1α,25-dihydroxyvitamin D_3, 19-nor-1α, 25-dihydroxyvitamin D_2, and 1α-hydroxyvitamin D_2 or 1α,25-dihydroxyvitamin D_2 on bone formation and resorption employing a mouse calvariae bone primary organ culture system and a Vitamin D/calcium-restricted rat model. Our results show that Vitamin D analogs, such as 19-nor-1α, 25-dihydroxyvitamin D_2 are effective in inducing anabolic bone formation.

2. Materials and methods

2.1. Materials

1α,25-dihydroxyvitamin D_3 (1α, 25-$(OH)_2D_3$) and 19-nor-1α, 25-dihydroxyvitamin D_2 (19-nor-1α, 25-$(OH)_2D_2$) were from Abbott Laboratories. 1α-Hydroxyvitamin D_2 (1α-$(OH)D_2$) and 1α,25-dihydroxyvitamin D_2 (1α, 25-$(OH)_2D_2$) were purchased from Tetrionics (Madison, WI). Other reagents were of analytical grade. All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Collagen synthesis in calvariae

Calvariae were cultured in BGJb medium (Invitrogen, Gaithersburg, MD) containing 1% insulin-transferrin-selenium-X, 0.1% BSA, 1 mM proline and 0.6 mM ascorbic acid phosphate (Growth Medium), and treated with test agents for 6 days. The calvariae were then transferred to fresh Growth Medium containing 10 μCi/ml of [^{3}H]proline (37 Ci/ml; Amersham) and incubated for 3 h. Each piece of calvariae was dehydrated, weighed and homogenized in 0.3 ml of 0.2 N NaOH. After the pH was neutralized, the solubilized fraction was treated with purified collagenase (Worthington, LS005273) at 37 °C for 1 h and the mixture was precipitated by trichloroacetic acid. The radioactivity in the supernatant (collagenase digestible protein) and the pellet (non-collagen protein) were determined separately [14].

2.3. Realtime RT-PCR

The total RNA was isolated using Trizol (Invitrogen) followed by RNeasy Mini Kit (Qiagen). The amount of extracted RNA was quantified by measuring the absorbance at 260 nm and the purity of the RNA was checked by the ratio between the absorbance values at 260 and 280 nm. The PCR primers and the TaqMan fluorogenic probe mix were purchased from Applied Biosystems. The first strand cDNA synthesis (reverse transcription) and the PCR were performed in a one-step methodology using MyiQ Real-Time PCR Detection System (Bio-Rad). The reaction mixture (25 μl final volume) contained 100 ng total RNA, ThermoScript Reaction Mix with 0.4 mM of dNTPs and 6 mM $MgSO_4$, ThermoScript Plus/Platinum Taq enzyme mix (Invitrogen) and 200 nM forward and reverse primers/200 nM probe mix (Applied Biosystems). Reverse transcription was performed at 50 °C for 30 min. The activation step (5 min at 95 °C) was followed by PCR (15 s at 95 °C and 60 s at 60 °C for