Journal of Steroid Biochemistry & Molecular Biology 136 (2013) 178–182

Contents lists available at SciVerse ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Review

Eldecalcitol and calcitriol stimulates 'bone minimodeling,' focal bone formation without prior bone resorption, in rat trabecular bone

Hitoshi Saito[a,*], Satoshi Takeda[b], Norio Amizuka[c]

[a] Medical Science Department, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan
[b] Product Research Department, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan
[c] Division of Oral Health Science, Graduate School of Dental Medicine, Hokkaido University, Sapporo, Japan

ARTICLE INFO

Article history:
Received 10 July 2012
Received in revised form 2 October 2012
Accepted 4 October 2012

Keywords:
Bone formation
Bone histomorphometry
Bone minimodeling
Vitamin D_3

ABSTRACT

Vitamin D is known as a potent stimulator of bone resorption. The active form of vitamin D_3, calcitriol (1α,25-dihydroxyvitamin D_3), stimulates release of calcium (Ca) from bone in *ex vivo* organ culture, and treatment with large amounts of an active vitamin D_3 analog induces hypercalcemia and bone resorption in mice *in vivo*. Calcitriol strongly induces both receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) in osteoblasts *in vitro*. On the other hand, it has been reported that active vitamin D_3 inhibits bone resorption in various experimental animal models.

We previously showed that eldecalcitol [1α,25-dihydroxy-2β-(3-hydroxy-propyloxy)vitamin D_3; ED-71] suppresses bone resorption and increases bone mineral density (BMD) to a greater extent than alfacalcidol (1α-hydroxyvitamin D_3) in ovariectomized (OVX) rats *in vivo*.

To elucidate the histological events that follow administration of eldecalcitol compared to calcitriol, OVX rats were given either vehicle, eldecalcitol (10, 30, or 90 ng/kg), or calcitriol (33.3, 100, 300, or 900 ng/kg), and sham-operated control animals were given vehicle, 5-times per week for 12 weeks. The lumbar spine and femur were removed and processed for bone mineral density (BMD) assessments and the femur for histomorphometrical analyzes.

Both eldecalcitol and calcitriol increased the lumbar and femoral BMD in a dose dependent manner. Bone histomorphometry revealed that osteoclast surface (Oc.S/BS) and eroded surface (ES/BS) were dose-dependently suppressed in the trabecular region of the femur. Both calcitriol and eldecalcitol dose-dependently stimulated focal bone formation that started without prior bone resorption, a process known as bone minimodeling. Both reduction of bone resorption and stimulation of focal bone formation were more clearly observed in the eldecalcitol-treated rats than in the calcitriol-treated rats.

Taken together, these findings suggest that eldecalcitol is a more potent vitamin D_3 analog that stimulates focal bone formation (minimodeling) and suppresses bone resorption more strongly than does calcitriol.

This article is part of a Special Issue entitled 'Vitamin D Workshop'.

Contents

* Corresponding author at: Medical Science Department, Medical Affairs, Chugai Pharmaceutical Co., Ltd., 2-1-1 Nihonbashi-Muromachi, Chuo-ku, Tokyo 103-8324, Japan. Tel.: +81 3 3273 2430; fax: +81 3 3281 2625.
E-mail address: saitohts@chugai-pharm.co.jp (H. Saito).

0960-0760/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jsbmb.2012.10.004

1. Introduction

Vitamin D is known as a potent stimulator of bone resorption. Vitamin D treatment elevates serum calcium (Ca) in vitamin D-depleted rats fed a Ca-free diet, indicating that vitamin D is important for the release of Ca from bone [1]. The active form of vitamin D_3, calcitriol (1α,25-dihydroxyvitamin D_3), stimulates Ca release in *in vitro* bone tissue culture [2,3], and treatment with large amounts of a vitamin D analog induces hypercalcemia and bone resorption in mice *in vivo* [4]. Calcitriol strongly induces both receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) in osteoblasts *in vitro*. Because RANKL and M-CSF are indispensable factors for the formation and survival of osteoclasts [2], it is generally accepted that vitamin D induces bone resorption by stimulating RANKL and M-CSF expression in bone.

On the other hand, it has also been reported that vitamin D inhibits bone resorption in various experimental animal models such as ovariectomized (OVX) rats and mice and orchiectomized rats [5–8]. Calcitriol inhibits osteoclast formation through suppressing c-Fos protein in osteoclast precursor cells [9]. Calcitriol suppresses the expression of nuclear factor of activated T cells c1 (NFATc1), a key regulator of osteoclast formation, by upregulating IFN-β in osteoclasts [10]. In thyroparathyroidectomized rats infused with parathyroid hormone (PTH), calcitriol inhibits PTH-induced bone resorption at a physiological dose and stimulates bone resorption at a toxic dose [11]. Taken together, these results indicate that vitamin D possesses opposing actions in both stimulating and inhibiting bone resorption.

We have previously shown that eldecalcitol [1α,25-dihydroxy-2β-(3-hydroxy-propyloxy)vitamin D_3; ED-71] suppresses bone resorption and increases bone mineral density (BMD) to a greater extent than alfacalcidol (1α-hydroxyvitamin D_3) in OVX rats *in vivo* [12]. A randomized, double-blind, placebo-controlled clinical trial revealed that treatment of osteoporotic patients with 0.75 μg/day of eldecalcitol for 12 months decreased urinary NTX (a bone resorption marker) by 20% from baseline levels, and increased lumbar and hip bone mineral density (BMD) by 3.4% and 1.5%, respectively, compared with the placebo group [13]. This effect of eldecalcitol on BMD was stronger than any previous results using alfacalcidol or calcitriol [14–16], and was observed without sustained hypercalcemia or hypercalciuria. Eldecalcitol has been approved for the treatment of osteoporosis in Japan.

In agreement with previous reports on the action of vitamin D analogs, we have shown by histological and histomorphometrical analyzes that eldecalcitol reduces osteoclast numbers and diminishes osteoclastic activity/function without promoting osteoclast apoptosis in OVX rats [12,17,18]. We have also reported that bone specimens from eldecalcitol-treated OVX rats show the ubiquitous presence of "bud-like" or "bouton-like" bone formation patterns characteristic of bone minimodeling, which is seen when new bone is deposited on previously quiescent surfaces and therefore features smooth cement lines [19]. Despite this uncommon pattern of bone formation characterized by the presence of smooth cement lines, active osteoblasts lying on the site of bone minimodeling were capable of synthesizing normal osteoids, and the mineralization process was intact. Immunohistochemistry for alkaline phosphatase (ALP) and proliferating cell nuclear antigen (PCNA) demonstrated that eldecalcitol promotes osteoblastic differentiation while suppressing osteoblast proliferation.

In the present study, we quantitatively evaluated the activity of bone minimodeling by a bone histomorphometrical approach, and compared the bone-forming activity of eldecalcitol with that of calcitriol in the bones of OVX rats.

2. Materials and methods

2.1. Reagents

Eldecalcitol was synthesized by Chugai Pharmaceutical (Tokyo, Japan). Calcitriol was purchased from Wako Pure Chemical Industries (Tokyo, Japan). Each compound was dissolved in a small amount of ethanol and diluted more than 1000-fold with medium chain triglyceride (MCT). MCT was used as the vehicle.

2.2. Animals

Seventy-two, 8-month-old female Sprague-Dawley rats were obtained from Charles River Laboratories Japan (Yokohama, Japan) and acclimated for 12 weeks under standard laboratory conditions. Rats were fed *ad libitum* with standard rodent chow (CE-2; CLEA Japan, Tokyo, Japan) and allowed free access to tap water.

Eight animals were sham-operated. Sixty-four animals were bilaterally ovariectomized (OVX) and then divided into 8 groups ($n = 8$ in each group). Each group of OVX animals was administered calcitriol (33.3, 100, 300, or 900 ng/kg body weight) or eldecalcitol (10, 30, or 90 ng/kg body weight) 5 times a week for 12 weeks by oral gavage. MCT was given to the sham-operated control animals and the OVX control animals in the same fashion. At 7 and 2 days prior to necropsy, tetracycline (20 mg/kg body weight) and calcein (6 mg/kg of body weight), respectively, were injected subcutaneously for bone labeling. The lumbar vertebrae (L2–L5) and the right femur were excised and stored in 70% ethanol.

The animal studies were approved by the Institutional Animal Care and Use Committee of Chugai Pharmaceutical Co., Ltd. and were performed in accordance with the ethics criteria contained in the bylaws of the Institutional Animal Care and Use Committee.

2.3. Bone mineral density and bone histomorphometry

The BMD of the second through fifth lumbar vertebra (L2–L5) and of the femur were measured by dual-energy X-ray absorptiometry (DXA: DCS-600EX; Aloka, Tokyo, Japan).

Bone histomorphometry was performed on trabecular bone of the distal metaphysis of the right femur. Femoral bones fixed in 70% ethanol were stained with Villanueva bone stain. After dehydration with ethanol, the specimens were defatted and embedded in methyl methacrylate, and 5-μm thick, undecalcified trabecular bone sections were prepared. Histomorphometrical parameters of the bone specimens were evaluated by using the Histomorphometric System (System Supply, Ina, Japan) attached to a microscope equipped with bright and epifluorescence illumination. This study uses the standard nomenclature and units of the American Society