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Review

Eldecalcitol improves mechanical strength of cortical bones by stimulating the periosteal bone formation in the senescence-accelerated SAM/P6 mice – A comparison with alfacalcidol

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ABSTRACT

Eldecalcitol (ELD), a 2β-hydroxypropyloxy derivative of $1\alpha,25(OH)_2D_3$, is a potent inhibitor of bone resorption that has demonstrated a greater effect at reducing the risk of fracture in osteoporotic patients than alfacalcidol (ALF). In the present study, we used the senescence-accelerated mouse strain P6 (SAM/P6), which has low bone mass caused by osteoblast dysfunction, to evaluate the effect of ELD on cortical bone in comparison with ALF. Four-month-old SAM/P6 mice were given either ELD (0.025 or 0.05 μg/kg) or ALF (0.2 or 0.4 μg/kg) by oral gavage 5 times/week for 6 weeks. Both ELD and ALF increased serum calcium (Ca) in a dose-dependent manner. Serum Ca levels in the ELD 0.05 μg/kg group were comparable to those of the ALF 0.2 μg/kg group. ELD 0.05 μg/kg significantly improved the bone biomechanical properties of the femur compared with the vehicle control group ($p < 0.001$) and the ALF 0.2 μg/kg group ($p < 0.05$) evaluated by 3-point bending test. The cortical area of the mid-femur in the ELD 0.05 μg/kg group but not the ALF 0.2 μg/kg group was significantly higher than those of the vehicle control group ($p < 0.001$). Bone histomorphometry revealed that in the femoral endocortical surface, the suppression of bone resorption parameters (N.Oc/BS) and bone formation parameters (MS/BS) by ELD (0.05 μg/kg) was greater than that by ALF (0.2 μg/kg). In contrast, in the femoral periosteal surface, ELD 0.05 μg/kg significantly increased bone formation parameters (BFR/BS, MS/BS) compared with the vehicle control group ($p < 0.05$, $p < 0.01$, respectively), whereas ALF 0.2 μg/kg did not alter these parameters. These results indicate that ELD improved the biomechanical properties of femoral cortical bone not only by inhibiting endocortical bone resorption but also by stimulating the periosteal bone formation in SAM/P6 mice.

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Contents

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1. Introduction

Eldecalcitol (ELD), a 2β-hydroxypropyloxy derivative of 1α,25(OH)$_2$D$_3$, calcitriol, is a potent inhibitor of bone resorption that has shown greater effects at increasing bone mineral density (BMD) and reducing the risk of bone fractures in osteoporotic patients than an existing active vitamin D$_3$ analog, alfacalcidol (ALF), in a large-scale randomized controlled study [1].

The binding activity of ELD to the serum vitamin D binding protein (DBP) is greater than that of 1α,25(OH)$_2$D$_3$ [2]. This characteristic contributes to its longer half-life in the circulation.

An in vivo study of ovariectomized rats showed that by strongly suppressing bone resorption, ELD was able to increase BMD by a greater amount than ALF [3]. ELD decreased urinary excretion of deoxypyridinoline and also decreased bone resorption parameters (eroded surface, osteoclast surface and osteoclast number) of a bone histomorphometrical analysis. Meanwhile, some studies with various rat models have indicated that ELD is able to maintain bone formation [3–6].

We have reported that 1α,25(OH)$_2$D$_3$ and ELD dose-dependently stimulated focal bone formation (bone minimodeling) of trabecular bone in the femoral metaphysis of rats [7]. Bone minimodeling is characterized by new bone formation without prior bone resorption. Bone is formed on previously quiescent surfaces, and therefore features smooth cement lines. The potency of ELD to induce bone minimodeling was reported to be approximately 10 times that of 1α,25(OH)$_2$D$_3$ [8].

The senescence-accelerated mouse strain P6 (SAM/P6) has low bone mass caused by osteoblast dysfunction [9], and has corresponding defects in endocortical mineralizing surface and bone marrow. The bones of SAM/P6 are weak and brittle because of defects in the bone matrix. These defects are attributed primarily to poorer organization of collagen fibers and reduced collagen content [10]. In contrast, these defects do not affect periosteal bone formation, and SAM/P6 mice have normal to enhanced bone formation on the periosteal surface.

In the present study, we evaluated the effect of ELD on cortical bone in the SAM/P6 mice in comparison with ALF.

2. Materials and methods

2.1. Reagents

Eldecalcitol [ELD: 2β-(3-hydroxypropyloxy)-1α, 25-dihydroxyvitamin D$_3$] and alfacalcidol (ALF: 1α-hydroxyvitamin D$_3$) were synthesized by Chugai Pharmaceutical Co., Ltd. Each compound was dissolved in a small amount of ethanol and diluted more than 1000-fold with medium chain triglyceride (MCT; O.D.O.-C, Nisshin Seiyu, Tokyo, Japan) to a predetermined concentration, then stored in the dark until use. MCT was used as the vehicle.

2.2. Animals

The animal studies were approved by the Institutional Animal Care and Use Committee of Chugai Pharmaceutical Co., Ltd., and were performed in accordance with ethics criteria contained in the bylaws of the Institutional Animal Care and Use Committee. Four-month-old SAM/R1 mice as a control strain and SAM/P6 mice were obtained from Japan SLC, Inc., (Ibaraki, Japan) and acclimated for 2 weeks under standard laboratory conditions. Mice were allowed free access to tap water and standard rodent chow (CE-2: CLEA Japan, Inc., Tokyo, Japan) in individual cages. SAM/P6 mice were randomly divided into 5 groups (n = 7-11). Each group of SAM/P6 mice was administered MCT, ELD (0.025, 0.05 μg/kg body weight) or ALF (0.2, 0.4 μg/kg body weight) 5 times/week for 6 weeks by oral gavage. MCT was also given to the SAM/R1 mice. At 7 days and 2 days prior to necropsy, calcein (8 mg/kg body weight) was injected subcutaneously for bone labeling. Both femurs were excised, and serum and urine samples were collected after the treatment. The right femur was stored in 70% ethanol for BMD measurement and bone histomorphometry. The left femur was frozen at −80 °C for measurement of bone strength. Serum samples were also frozen at −80 °C for biochemical analysis.

2.3. Measurements of serum Ca, femoral BMD and bone strength

Serum Ca concentrations (mg/dL) were measured using an automatic analyzer (Hitachi 7070; Hitachi, Ltd., Tokyo, Japan). The average BMD (mg/cm^2) of the right femur was analyzed by single-energy X-ray absorptiometry (DCS-600EX; Aloka Co., Ltd., Tokyo, Japan), starting the scanning at the most proximal area and ending at the most distal area. The left femur was used to measure the mechanical strength by the 3-point bending test [11] using a mechanical strength analyzer (TK-252CC, Muromachi Kikai, Co., Ltd., Japan). In brief, the left femur was placed on a special holding device with supports located at a distance of 6 mm apart. A bending force was applied with the cross head at a speed of 10 mm/min, until a fracture occurred. From the load-deformation curve, the breaking strength (max load: N) was obtained.

2.4. Bone histomorphometry

Bone histomorphometry was performed on the right femur. Femoral bone fixed in 70% ethanol was stained according to the method of Villanueva [12]. After dehydration with ethanol and acetone, the specimens were defatted and embedded in methyl methacrylate. For the femoral diaphysis, 20-30 μm thick cross-cut ground sections were obtained with a micro-grinding machine system (KG4000, EXAKT, Germany) and prepared for measurement. The image of the specimen, observed under a fluorescence microscope and recorded with a video camera, was processed using a plotter (Cosmozome 1SA; Nikon, Tokyo, Japan) to measure the primary parameters on the endocortical and periosteal perimeters: total cross-sectional area (mm^2), marrow area (mm^2), cortical bone area (mm^2), outer perimeter (mm), inner perimeter (mm), bone surface (BS, μm), osteoclast number (N.Oc), single labeled surface (sLS, μm), double labeled surface (dLS, μm), and double labeled thickness (L.Th, μm). From these primary parameters, the following parameters were calculated for kinetic parameters: number of osteoclasts per mm trabecular surface (N.Oc/BS,/mm), bone formation rate (BFR/BS, μm^3/μm^2/year), mineralizing surface (MS/BS, %) and mineral apposition rate (MAR, μm/day). Nomenclature, symbols, and units used in this study are those described in the Report of the American Society for Bone and Mineral Research Nomenclature Committee [13].

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