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# Effects of combined treatment with eldecalcitol and alendronate on bone mass, mechanical properties, and bone histomorphometry in ovariectomized rats: A comparison with alfacalcidol and alendronate

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ABSTRACT

Eldecalcitol (ELD), a 2β-hydroxypropyloxy derivative of 1α,25 (OH) 2D3, inhibits bone resorption more potently than alfacalcidol (ALF) while maintaining osteoblastic function in an ovariectomized (OVX) osteoporosis rat model. Alendronate (ALN), which is the most common bisphosphonate used for the treatment of osteoporosis, increases the bone mineral density (BMD) by suppressing bone resorption. In this study, we investigated the effects of combination treatments with ELD and ALN or with ALF and ALN on bone mass and strength in OVX rats. Seventy female rats, 32 weeks old, were assigned to seven groups: (1) a sham-operated control group; (2) an OVX-control group; (3) an ELD group; (4) an ALF group; (5) an ALN group; (6) an ELD + ALN group; and (7) an ALF + ALN group. OVX rats were orally treated with ELD (0.015 μg/kg), ALF (0.0375 μg/kg), or ALN (0.2 mg/kg) daily for 12 weeks. In both the lumbar spine and the femur, ELD and ALF monotherapy significantly increased the BMD, and ELD + ALN and ALF + ALN significantly increased the BMD, compared with ALN monotherapy, as an additive effect. In particular, ELD + ALN resulted in a significantly higher BMD than ALF + ALN in the femur. On mechanical testing of the lumbar spine, ELD and ALF monotherapy significantly increased the ultimate load, and ELD + ALN and ALF + ALN significantly increased the ultimate load compared with ALN monotherapy. In the femur, ELD, ELD + ALN, and ALF + ALN treatment significantly increased the ultimate load, compared with the OVX-control group, and ELD + ALN resulted in a significantly higher ultimate load than ALN monotherapy. A histomorphometric analysis showed that ELD monotherapy and ELD + ALN combination therapy had a potent inhibitory effect on bone resorption parameters (osteoclast surface and eroded surface), while maintaining bone formation parameters (osteoblast surface and osteoid surface). By contrast, ALF and ALF + ALN significantly lowered the histological parameters of both bone resorption and formation. These results suggested that ELD or ALF used in combination with ALN has therapeutic advantages over ALN monotherapy, with ELD + ALN combination treatment producing an especially beneficial anti-osteoporotic effect by inhibiting osteoclastic bone resorption and maintaining osteoblastic function, compared with ALF + ALN combination treatment.



## Introduction

Osteoporosis is characterized by a decreasing bone mass with age and microarchitectural disturbances in the bone tissue, with a consequent increase in bone fragility and susceptibility to fracture [1]. The incidence of osteoporosis-related fractures seems to be increasing, indicating that osteoporosis is becoming a major health problem worldwide [2]. Postmenopausal osteoporosis is induced by accelerated bone resorption and a systemic calcium (Ca) imbalance caused by estrogen deficiency as a result of menopause. Much attention has been paid to vitamin D. Inadequate 25-hydroxy-vitamin D (25OHD) levels are common in elderly people, as their ability to synthesize vitamin D in the skin is believed to be reduced, compared with younger people. Vitamin D insufficiency is a particular concern in patients with fragility fractures, such as hip fractures [3,4].

Vitamin D is associated with the treatment of some Ca- and bone-related diseases, and vitamin D3 [1α,25 $(OH)_2D_3$] derivatives, including the prodrug alfacalcidol (ALF) and falecalcitriol [5], have been developed as therapeutic drugs for Ca-related and bone-related diseases including osteomalacia and hyperparathyroidism. Moreover, ALF and 1α,25 $(OH)_2D_3$ are widely used as a treatment for osteoporosis, and evidence that 1α,25 $(OH)_2D_3$ reduce hip fractures in postmenopausal osteoporosis has been reported [6]. Preclinical studies have shown that active vitamin D metabolites can prevent estrogen deficiency-induced bone loss by suppressing bone resorption [7–10].

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Some reports have suggested that vitamin D metabolites affect bone formation, increasing osteoblast activity by augmenting the proliferation of osteoblasts [11,12]. ELD is a novel analog of 1α,25 $(OH)_2D_3$, bearing a hydroxypropoxy substituent at the 2β-position. ELD has a greater activity than ALF in suppressing bone resorption as well as increasing BMD and lowers bone resorption without reducing bone formation in an ovariectomized rat model of osteoporosis [13,14]. Also, in human osteoporosis, ELD has been proven to suppress bone resorption markers and to increase the lumbar and hip BMD after 1 year of treatment [15]. In a recent randomized, double-blind study, ELD was shown to have a greater preventative effect than ALF on vertebral and wrist fractures in osteoporotic patients under conditions of vitamin D supplementation, with a safety profile similar to that of ALF [16].

Bisphosphonates (BPs) such as alendronate (ALN) and risedronate are widely used in the treatment of postmenopausal osteoporosis [17,18]. BPs increase the BMD of the spine, hip, and total body by inhibiting bone resorption, leading to the suppression of bone turnover and reducing the incidence of vertebral and non-vertebral fractures [19,20]. They also prevent estrogen deficiency-induced bone loss in animals and have a suppressive effect on the bone resorption of mature osteoclasts and the induction of osteoclast apoptosis [21,22]. Previous reports have shown that combination therapies with vitamin D analogs (ALF) and BPs may be more effective than BP monotherapy for preventing bone loss and lowering the incidence of fractures. Recently, the combination treatment of ELD and ALN was shown to be more beneficial, compared with ALN monotherapy, with regard to bone loss and bone strength in ovariectomized rats [23]. On the other hand, the study comparing the efficacy of ELD and ALF used in combination with ALN has not been previously reported, although ELD monotherapy offered advantages over ALF treatment in clinical and preclinical studies.

In this study, we investigated the effects of combination treatments with ELD and ALN or with ALF and ALN on bone mass, bone strength, bone turnover markers, and histomorphometric parameters in OVX rats.

## Materials and methods

### *Reagent*

1α,25-Dihydroxy-2β-(3-hydroxypropyloxy) vitamin D3 (eldecalcitol: ELD) and 1α-hydroxyvitamin D3 (alfacalcidol: ALF) were provided by Chugai Pharmaceutical Co., Ltd. Both drugs were dissolved in medium-chain triglyceride (MCT). Alendronate (ALN) was purchased from Sigma-Aldrich Co. (St. Louis, MO) and was dissolved in sterile physiological saline.

### *Animals*

Seven-month-old, female Wistar-Imamichi rats were purchased from the Institute for Animal Reproduction (Ibaraki, Japan) and were acclimated until 8 months of age under standard laboratory conditions, at 23 ± 2 °C and a humidity of 40%–70%. The rats were maintained in individual stainless wire cages, were fed a standard rat chow (CE2; Clea Japan Inc., Tokyo, Japan), and were given tap water.

### *Study design*

At 8 months of age, the animals were subjected to bilateral OVX or a sham operation (Sham) under anesthesia. The rats were divided into 7 groups as shown in Table 1. Animals received daily doses of vehicles (PBS and MCT, groups 1 and 2), 0.015 μg/kg of ELD and PBS (group 3), 0.0375 μg/kg of ALF and PBS (group 4), 0.2 mg of ALN and MCT (group 5), a combination of ELD and ALN (group 6), or a combination of ALF and ALN (group 7). All the compounds and their vehicles were orally administered daily for 12 weeks at a dose volume of 1 mL/kg after the operation. The dosage level of each drug was determined based on the clinical doses (0.015 μg/kg, 0.0375 μg/kg, and 0.2 mg/kg for ELD, ALF, and ALN, respectively). ALN was administered after 2 h of fasting to prevent interference with the ALN absorption. ELD or ALF was given 2 h after ALN dosing. The body weight of each animal was measured once a week until the final day of administration. A 24-h urine sample was collected after the 12th week treatment and was stored at −80 °C until analysis. On the day after the final drug administration, the rats were anesthetized under isoflurane and the blood was collected from the abdominal aorta into a syringe. The sample was then centrifuged at 1600 × *g* for 10 min at 4 °C to separate the plasma and stored at −80 °C. After collecting the blood sample, the lumbar vertebrae (L2–L5) and bilateral femurs were removed. The L5 vertebra and the left femur were prepared for the mechanical tests. The L2–L4 vertebrae and right femur were used for the BMD measurements, and the L3 vertebra was used to analyze the bone histomorphometry parameters. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Taisho Pharmaceutical Co., Ltd.

**Table 1**
Study design.

| Group | N | Surgery | Treatment | Dose |
|---|---|---|---|---|
| 1 | 10 | SHAM | Vehicle | – |
| 2 | 10 | OVX | Vehicle | – |
| 3 | 10 | OVX | ELD | 0.015 μg/kg |
| 4 | 10 | OVX | ALF | 0.0375 μg/kg |
| 5 | 10 | OVX | ALN | 0.2 mg/kg |
| 6 | 10 | OVX | ELD+ALN | 0.015 μg/kg, 0.2 mg/kg |
| 7 | 10 | OVX | ALF+ALN | 0.0375 μg/kg, 0.2 mg/kg |

ELD: eldecalcitol, ALF: alfacalcidol, ALN: alendronate.

### *Biochemical analysis*

The concentration of Ca, inorganic phosphorus (P), and alkaline phosphatase (ALP) in the serum and the creatinine (CRE) level in the urine were measured using an autoanalyzer (Hitachi 7170; Hitachi Co., Ltd., Tokyo, Japan). The serum osteocalcin level was measured using an ELISA kit from GE Healthcare according to the manufacturer's instructions (Little Chalfont, UK). The urine deoxypyridinoline (D-Pyr) level was measured using an ELISA according to the manufacturer's instructions (DS Pharma Biomedical Co., Ltd., Osaka, Japan).

### *Measurement of mechanical properties*

A mechanical strength analyzer (TK-252C; Muromachi Kikai Co., Ltd., Tokyo, Japan) was used for the biomechanical tests. An axial compression test was performed to analyze the mechanical strength of the lumbar vertebra (L5) [24]. For the compression test, planoparallel surfaces were obtained by removing the cranial and caudal ends of the vertebral specimen. From the vertebral body, a central cylinder with planoparallel ends and a height of approximately 5 mm was obtained. A craniocaudal compression force was applied to the specimen using a steel disk at a deformation rate of 2.5 mm/min. A three-point bending test was performed to analyze the mechanical strength of the midshaft of the femur [25]. The left femur was placed on a special holding device with supports located 12 mm apart. A bending force was applied with a cross head at the rate of 20 mm/min until a fracture occurred. For both bones, the ultimate compressive load (N) was calculated as a representative mechanical property directly from the load-displacement curve.

### *Measurement of bone mineral density*

The average BMD ($mg/cm^2$) of the lumbar vertebrae (L2–L4) was measured using DXA (DCS-600EX-IIIR; Alock Co., Ltd., Tokyo, Japan).