Effects of Alendronate and Alfacalcidol on the Femoral Bone Mass and Bone Strength in Orchidectomized Rats

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Abstract

The purpose of the present study was to compare the effects of alendronate (ALN) and alfacalcidol (ALF) on the femoral bone mass and bone strength in orchidectomized rats and to clarify the skeletal benefits of combined administration of ALN and ALF. Fifty male Sprague-Dawley rats, 3 months of age, were randomized by the stratified weight method into five groups: the age-matched control (CON), orchidectomy (ORX), ORX + ALN (2.5 µg/kg, s.c., 5 times a week), ORX + ALF (0.1 µg/kg, p.o., 5 times a week), and ORX + ALN + ALF groups. After 12 weeks of feeding, the femoral distal metaphysis and mid-diaphysis were processed for peripheral quantitative tomographic analysis and biomechanical testing. In the femoral distal metaphysis, ALN prevented the ORX-induced reduction in the trabecular volumetric bone mineral density (vBMD) and breaking energy, and ALF prevented the ORX-induced reduction in the trabecular vBMD and increased the breaking energy to values above those observed in the CON group. Both ALN and ALF increased the maximum load to values above those observed in the ORX group. The improvements in parameters described above were more pronounced when ALN and ALF were administered in combination. In the femoral mid-diaphysis, on the other hand, ALN did not significantly affect the cortical bone parameters, whereas ALF increased the cortical area and maximum load to values above those observed in the ORX group. Furthermore, no apparent benefit of combined administration of ALN and ALF was observed. These findings suggest differential effects of ALN and ALF on femoral bone mass and the beneficial effects of combined administration of ALN and ALF on the trabecular bone of the femur in ORX rats.

Key Words: orchidectomy, BMD, alendronate, alfacalcidol, rat

Introduction

Orchidectomized rats have been used to clarify the role of testosterone in skeletal growth during the period of linear growth in males and in maintenance of the skeletal mass in the later stages of life (1, 2, 7). Because testosterone deficiency, caused by orchidectomy (ORX), has been reported to induce high-turnover cancellous osteopenia and loss of cortical bone gain with decreased periosteal bone formation and cortical porosity in rats (5, 10, 19), both antiresorptive and anabolic agents could be candidates for treatment of the skeletal changes caused by ORX in rats. It is also expected that combined administration of antiresorptive and anabolic agents might exert complementary effects in improving the bone mass and bone strength in ORX rats.
Alendronate (ALN) and alfacalcidol (ALF) are commercially available in Japan. ALN is known to inhibit osteoclast-mediated bone resorption (20), while ALF exerts both anabolic and antiresorptive effects on the skeleton (13-16, 21, 22). ALN has been reported to reduce the bone turnover, prevent loss of bone density, and preserve or increase the bone strength of the femur (3, 8). However, very few studies have reported on the effects of ALF on the bone mass and bone density, and preserve or increase the bone strength of ORX rats. The purpose of the present study was to compare the effects of ALN and ALF on bone strength in ORX rats and to clarify the skeletal benefits of combined administration of ALN and ALF.

Materials and Methods

Treatment of Animals

Fifty male Sprague-Dawley (SD) rats, 3 months of age, were purchased from Hilltop Lab. Animals, Inc. (Scottsdale, PA, USA). The animals were housed under local vivarium conditions (temperature 23.8°C and 12-h on/off light cycle), fed a pelleted standard chow diet containing 1.36% calcium and 2400 IU/kg of vitamin D (Rodent Diet 8604, Harlan Teklad, Madison, WI, USA), and had free access to water. Following a one-week adaptation period in their new environment, the rats were randomized by the stratified weight method into five groups of 10 rats each according to the treatment schedule, as follows: the age-matched control (CON), ORX, ORX + ALN, ORX + ALF, and ORX + ALN + ALF groups. ORX was performed just after the grouping of the rats, and drug administration to the ORX rats was started one day after the surgery. ALN (Merck, NJ, USA) was dissolved in 0.1 ml of sterile saline and administered by subcutaneous injection at the dose of 2.5 µg/kg body weight five times a week. ALF (Teijin Pharma, Tokyo, Japan) was dissolved in 0.1 ml of PBS containing 0.25% ethanol and 0.1% Tween 20 and administered by gavage deep into the mouth at the dose of 0.1 µg/kg body weight five times a week. These doses of ALN and ALF were considered to be effective in rats based on previously published data (13, 17, 21, 22). The body weight of the rats was monitored weekly, and the total experimental period was 12 weeks. The study was carried out at Winthrop-University Hospital, and the animals were maintained according to the National Institutes of Health (NIH) Guidelines for Care and Use of Laboratory Animals. All the animal experimental protocols were approved by the Laboratory Animal Care Committee of Winthrop-University Hospital.

Preparation of Specimens

At the end of the experimental period of 12 weeks, all the rats were anesthetized by intraperitoneal injection of 80 mg/kg ketamine + 12 mg/kg xylazine, and sacrificed by exsanguination. The left femurs and right tibiae were removed. The femurs were stored in a freezer (-70°C) and processed later for measurements of the femoral length, peripheral quantitative tomographic analysis and biomechanical testing. The tibiae were used to obtain bone histomorphometric images. The bones were fixed overnight in 40% cold ethanol and then cut into three parts using an Isomet saw (Buehler, Lake Bluff, IL, USA). The tibial proximal metaphyses and tibial mid-diaphyses were stained with Van Kossa Stain for 5 days. The specimens were then dehydrated sequentially in ascending concentrations of ethanol (70%, 95%, and 100%) and xylene and then embedded in methyl methacrylate (EM Science, Gibbstown, NJ, USA) at 4°C. Cross-sections of the tibial diaphysis just proximal to the tibio-fibular junction were cut at 10-µm thickness using a microtome (Leica RM2155; Leica Inc., Nussloch, Germany), and the thickness of each cross-sectional specimen was determined with an Inspectors’ Dial Bench Gauge (L.S. Starrett, Athol, MA, USA). Frontal sections of the tibial proximal metaphysis were cut at 5-µm thickness using a microtome (Leica RM2155; Leica Inc., Nussloch, Germany), transferred onto chromium-gelatin-coated slides, dried overnight under pressure at 42°C, and coverslipped with Eukitt mounting medium (Calibrated Instruments, Hawthorne, NY, USA).

Measurement of the Femoral Length and Peripheral Quantitative Tomographic Analysis

Just after measurement of the length of the femur using the dial calipers, the distal metaphysis and mid-diaphysis of this bone were scanned by peripheral quantitative tomography (pQCT; Norland/Stratec XCT Research SA; Stratec Medizintechnic GmbH, Pforzheim, Germany) in 50% ethanol/saline. The bones were placed horizontally inside a glass tube and scanned at a voxel size of 0.12 mm. The scan line was adjusted using the scout view of the pQCT system. Skeletal sites 3 mm and 14 mm proximal to the distal growth plate were scanned. For the analysis, a threshold of 395 mg/cm³ in contour mode 2 was used to separate the bone areas from the marrow regions. To separate the cortical areas from the trabecular areas, a constant threshold of 690 mg/cm³ was used. Volumetric bone mineral density (vBMD, mg/cm³), the periosteal and endocortical bone surface, cortical area, and the x-axis and polar strain stress index (SSI) were calculated. The x-axis and polar SSI reflect the bone strength determined by the three-point bending and torsion tests, respectively (6, 18).

Biomechanical Testing

Immediately after the PQCT analysis, the bone...
strength of the mid-diaphysis of the femur was evaluated by the three-point bending test. A load was applied midway between two supports placed 15 mm apart. The femur was positioned so that the loading point was at the center of the femoral mid-diaphysis and bending occurred about the medial-lateral axis. The specimens were tested in a saline bath at 37°C. Each specimen was submerged in the saline bath for about 3 min before the testing, to allow temperature equilibration. Load-displacement curves were recorded at a crosshead speed of 20 mm/min using a materials-testing machine (MZ500D; Maruto, Co., Ltd., Tokyo, Japan). The parameters analyzed were maximum load, stiffness, and breaking energy.

Just after the three-point bending test of the femoral mid-diaphysis, the distal metaphysis of the femur was isolated over a length of 10 mm from the joint surface of the femoral condyle. The bone strength of this segment was then measured by the compression test. Compressive load was applied with a rectangular parallelepiped crosshead (length 2 cm, width 2 cm, height 1 cm) on the femoral distal metaphysis, from the lateral aspect to the medial aspect. The specimens were tested in a saline bath at 37°C. Each specimen was submerged in the saline bath for about 3 min before the testing, to allow temperature equilibration. Load-displacement curves were recorded at a crosshead speed of 10 mm/min and compression depth of 2.5 mm, using a materials-testing machine (MZ500D; Maruto, Co., Ltd., Tokyo, Japan). The parameters analyzed were maximum load, stiffness, and breaking energy.

### Statistical Analysis

All the data were expressed as means ± standard deviation (SD). Multiple comparisons of the data among the groups were performed by analysis of variance (ANOVA) using Fisher’s protected least significant difference (PLSD) test. All the statistical analyses were performed using the Stat View J-5.0 program on a Macintosh computer. A significance level of \( P < 0.05 \) was used for all the comparisons.

### Results

#### Body Weight and Femoral Length

Table 1 shows the body weight and femoral length of the rats. The initial body weight did not differ significantly among the groups. The final body weight and femoral length also did not differ significantly among the groups.

#### \( vBMD, SSI \) and Bone Strength of the Femoral Distal Metaphysis

Figures 1 and 2 show the \( vBMD \) and SSI as measured by pQCT and the bone strength of the femoral distal metaphysis as measured by the compression test, respectively. As compared with the findings in the CON group, ORX caused reduction in the trabecular \( vBMD, SSI \) (x-axis) and breaking energy. ALN prevented this ORX-induced reduction in the trabecular \( vBMD, SSI \) (x-axis) and breaking energy, while ALF prevented the reduction in the trabecular \( vBMD \) and even increased the SSI (x-axis) and breaking energy to values above those noted in the CON group. Both ALN and ALF increased the total \( vBMD, SSI \) (polar) and maximum load to values above those observed in the ORX group, without significantly affecting the stiffness. The improvements of all of these parameters were more pronounced when ALN and ALF were administered in combination.
Fig. 1. Peripheral quantitative tomography analysis of the femoral distal metaphysis. Data are expressed as means ± SD. ANOVA with Fisher’s PLSD test was used to compare the data among the groups. a, significant vs. CON; b, significant vs. ORX; c, significant vs. ALN; d, significant vs. ALF. CON: age-matched control, ORX: orchidectomy, ALN: ORX+administration of alendronate, ALF: ORX+administration of alfacalcidol, ALN+ALF: ORX+administration of alendronate and alfacalcidol. vBMD: volumetric bone mineral density, SSI: stress strain index.

Fig. 2. Bone strength of the femoral distal metaphysis. Data are expressed as means ± SD. ANOVA with Fisher’s PLSD test was used to compare the data among the groups. a, significant vs. CON; b, significant vs. ORX; c, significant vs. ALN; d, significant vs. ALF. CON: age-matched control, ORX: orchidectomy, ALN: ORX+administration of alendronate, ALF: ORX+administration of alfacalcidol, ALN+ALF: ORX+administration of alendronate and alfacalcidol.

vBMD, SSI, and Bone Strength of the Femoral Mid-Diaphysis

Figures 3 and 4 show the vBMD and SSI as measured by pQCT and the bone strength of the femoral mid-diaphysis as measured by the three-point bending test, respectively. ALN did not significantly affect any of the bone parameters, whereas ALF increased the cortical area and maximum load to values above those observed in the ORX group and decreased the endocortical circumference as compared with the value in the CON group. However, no apparent benefit of administration of ALN and ALF in combination was observed, except for an increase of the periosteal circumference and SSI (x-axis) as compared with the values noted in the ORX group.

Bone Histomorphometric Images of the Tibia

It is known that both the femoral and tibial mid-diaphyses are composed of cortical bone, whereas both the femoral distal and tibial proximal metaphyses are rich in trabecular bone. Fig. 5 shows the bone histomorphometric images of the tibial proximal metaphysis and tibial mid-diaphysis. The imaging findings support the results of the pQCT analysis of the femoral distal metaphysis and femoral mid-diaphysis. However, alterations in cortical bone parameters of the femoral mid-diaphysis were modest, and, therefore, no apparent difference was observed.
Discussion

The present study was conducted to compare the effects of ALN and ALF on the bone mass and bone strength of the femoral distal metaphysis and femoral mid-diaphysis in ORX rats and to clarify the skeletal benefits of combined administration of ALN and ALF. Both ALN and ALF improved trabecular vBMD and bone strength of the femoral distal metaphysis, and these improvements were more pronounced when the two agents were administered in combination. However, only ALF improved the cortical area and cortical bone strength of the femoral mid-diaphysis. Thus, we confirmed the differential effect of ALN and ALF on the femoral bone and the beneficial effects of combined administration of ALN and ALF on the trabecular bone of the femur in ORX rats.

Previously, we clarified, based on the findings in bone specimens obtained from 3 month-old male SD rats, that ORX induced cancellous osteopenia of the tibial proximal metaphysis as a result of increased bone resorption, and reduced maturation-related gains in the images of tibial mid-diaphysis.
in the femoral bone area, bone mineral content (BMC) and BMD, and also the cortical bone mass of the tibial diaphysis as a result of decreased periosteal bone formation (11). In the present study, ORX resulted in reductions in the trabecular vBMD, SSI (x-axis) and breaking energy of the femoral distal metaphysis, without significantly affecting the cortical bone parameters of the femoral mid-diaphysis. Thus, ORX-induced skeletal changes in terms of changes in the vBMD and bone strength might be more significant in the trabecular bone than in the cortical bone, probably because of the different bone turnover rate between the trabecular and cortical bone.

A few reports have shown that ALN reduces the bone turnover, prevents loss of the bone density, and preserves or increases the bone strength of the femur in ORX rats (3, 8). In the present study, ALN prevented the ORX-induced reduction in the trabecular vBMD, SSI (x-axis) and breaking energy, and even increased the SSI (polar) and maximum load to values above those noted in the ORX group in the femoral mid-diaphysis. Unlike ALN, ALF might have affected both the trabecular and cortical bone in the ORX rats and, therefore, have exerted a greater effect on the maximum load of the femoral distal metaphysis and the cortical area of the femoral mid-diaphysis, suggesting the greater efficacy of ALF on the trabecular and cortical bone in ORX rats. On the other hand, the beneficial effect of ALF on the cortical bone might be less pronounced than that on the trabecular bone.

Ito et al. reported the more pronounced effect of combined administration of ALN and ALF on the cancellous bone mass in the lumbar spine as compared with that of single administration of either agent alone in ovariectomized rats (9). In the present study, improvements of the trabecular vBMD, SSI (x-axis and polar), maximum load, stiffness and breaking energy in the femoral distal metaphysis induced by treatment were found to be more pronounced when ALN and ALF were administered in combination. Thus, ALN and ALF might exert greater beneficial effects on the trabecular vBMD and bone strength than either agent administered alone. There was a small benefit of combined administration of ALN and ALF in improving the cortical bone geometry and maximum load of the femoral mid-diaphysis. However, no apparent
benefit of combined administration of ALN and ALF was observed in the femoral mid-diaphysis, probably because the effect of ALF on the cortical bone might be modest in ORX rats.

In conclusion, the present study showed the effects of ALN on the femoral distal metaphysis and that of ALF on both the femoral distal metaphysis and mid-diaphysis in ORX rats. Combined administration of the two agents, as compared to single administration of either agent alone, appeared to have beneficial effects only on the femoral distal metaphysis. These findings suggest the differential effects of ALN and ALF on the femoral bone and the beneficial effects of combined administration of ALN and ALF on the trabecular bone of the femur in ORX rats.

References