Comparative Therapeutic Effects of Alendronate and Alfalcaldol on Cancellous and Cortical Bone Mass and Mechanical Properties in Ovariectomized Osteopenic Rats

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Summary The purpose of the present study was to compare the therapeutic effects of alendronate and alfalcaldol on the cancellous and cortical bone mass and mechanical properties in ovariectomized osteopenic rats in a head-to-head fashion. Twenty-five female Sprague-Dawley rats, 7 mo of age, were randomly divided by the stratified weight method into four groups: the sham-operated control (Sham) group and three ovariectomized groups treated with vehicle, alendronate (2.5 mg/kg, p.o., daily), or alfalcaldol (0.5 μg/kg, p.o., daily). Treatment was started 6 wk after the surgery and continued for 6 wk. At the end of the experiment, urinary deoxypyridinoline (DPD) and serum osteocalcin (OC) levels were evaluated, and cancellous and cortical bone histomorphometric analyses were performed for the proximal tibial metaphysis and tibial diaphysis, respectively. Alendronate prevented the elevation of the urinary DPD level induced by ovariectomy (OVX), and markedly decreased the serum OC level to below the value observed in the Sham group, while alfalcaldol prevented the elevation of the urinary DPD and serum OC levels induced by OVX. Alendronate increased the cancellous bone volume/total tissue volume (BV/TV) relative to the values observed in the OVX-Vehicle group by preventing the increases in the eroded surface/bone surface (ES/BS), osteoclast surface (OCs)/BS, and bone formation rate (BFR)/BS induced by OVX. However, it decreased the mineral apposition rate (MAR) in the ovariectomized osteopenic rats to below the value observed in the Sham group. It also prevented the increase in the marrow area (Ma Ar) caused by OVX. Alfalcaldol increased the BV/TV relative to the values observed in the OVX-Vehicle group by decreasing the ES/BS and OCs/BS, but maintaining the BFR/BS. The effect of alfalcaldol on the BV/TV was more pronounced than that of alendronate, despite the less pronounced suppression of OCs/BS by this drug in the ovariectomized osteopenic rats. In addition, this drug increased the cortical area (Ct Ar) and prevented the increase in the Ma Ar in the ovariectomized osteopenic rats by decreasing the endocortical ES/BS, and even increasing the endocortical BFR/BS. Furthermore, it also prevented the reduction in the maximum load of the femoral distal metaphysis in the ovariectomized osteopenic rats. Thus, the present study clearly showed that alendronate and alfalcaldol had differential therapeutic effects on the cancellous and cortical bone mass and mechanical properties in ovariectomized osteopenic rats.

Key Words ovariectomy, osteopenia, alendronate, alfalcaldol, therapy

Osteoporosis is recognized as a major public health problem, especially in postmenopausal women, because estrogen deficiency associated with menopause causes marked bone loss. Alendronate and alfalcaldol have been widely used for postmenopausal women with osteoporosis in Japan. The results of randomized controlled head-to-head trials suggest that alendronate at 5 mg/d is more effective than alfalcaldol at 1 μg/d at increasing the lumbar bone mineral density (BMD) and reducing the incidence of vertebral fractures in Japanese postmenopausal women with osteoporosis (1, 2). However, the effect of these drugs on the BMD and the incidence of fractures at skeletal sites predominant in cortical bone remains uncertain.

Several animal studies have reported on the therapeutic effects of alendronate and alfalcaldol on the bone mass and mechanical properties in ovariectomized osteopenic rats (3–5): Alendronate has been reported to preserve the trabecular bone area and bone strength of the lumbar spine (3), while alfalcaldol has...
been reported to increase the BMD of the femoral diaphysis (4), and the bone mineral content, BMD, and mechanical properties of the femur (5). However, there have been no animal studies, in which the effect of alendronate and alfacalcidol on bone were compared in a head-to-head fashion, and thus the differential effects of alendronate and alfacalcidol on the bone mass, bone formation and bone resorption as evaluated by bone histomorphometric analyses and mechanical properties in ovariectomized osteopenic rats remain to be clarified. We employed ovariectomized osteopenic rats as an animal model of postmenopausal osteoporosis and directly compared the effects of alendronate and alfacalcidol on the cancellous and cortical bone mass and mechanical properties.

MATERIALS AND METHODS

Treatment of animals. Twenty-five female Sprague-Dawley rats, 7 mo of age, were purchased from Charles River Japan (Kamagawa, Japan). They were fed a pelleted standard chow diet containing 1.25% calcium and 0.9% phosphorus (CRF-1; Oriental Yeast, Co., Ltd., Tokyo, Japan). The animals were housed under local vivarium conditions (temperature 23.3°C, humidity 55%, and 12 h on/off light cycle), with free access to water. After one week for adaptation to the new environment, the rats were randomized by the stratified weight method into the following four groups: sham-operation + vehicle (Sham) group (n = 5), bilateral ovariectomy (OVX) + Vehicle group (n = 5), OVX + Alendronate (2.5 mg/kg) group (n = 7), and OVX + Alfacalcidol (0.5 μg/kg) group (n = 8). The treatment with vehicle, alendronate, or alfacalcidol was started 6 wk after the surgery and continued for 6 wk. Bilateral OVX was performed under general anesthesia induced by intraperitoneal injection of 25–30 mg/kg pentobarbital sodium. Tablet forms of alendronate (Bonafon, Teijin Pharma, Tokyo, Japan) or alfacalcidol (One-alfa, Teijin Pharma, Tokyo, Japan) were pulverized, dissolved in 0.1 ml of sterile saline, and administered orally to the animals daily by gavage deep into the mouth. The dose of alendronate was determined according to the results of a previous study (6). The dose of alfacalcidol was determined so that the alfacalcidol/alendronate dose ratio was 1 μg/5 mg, based on the clinically used dose in Japan. This dose of alfacalcidol is sufficient for the drug to exert its effects on bone according to the results of previous studies (7–9). The body weight of the rats was monitored weekly, and the total duration of the experiment was 12 wk. The present study was carried out at the laboratory of Hamri Co., Ltd. (Ibaraki, Japan). The animals were maintained according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and the animal experiment protocols were approved by the Laboratory Animal Care Committee of Hamri Co., Ltd. (Ibaraki, Japan).

Preparation of the specimens. Urine samples were collected from all the rats over a 24-hr period, using metabolic cages, 6, 9, and 12 wk after the start of the experiment, and the specimens were stored at −20°C. All the rats were labeled with 25 mg/kg of tetracycline (Sigma Chemical, St. Louis, MI, USA) injected intramuscularly and 8 mg/kg of calcine (Sigma Chemical) injected subcutaneously, 9 d and 3 d, respectively, before being sacrificed. After 12 wk, the animals were sacrificed by exsanguination under anesthesia induced by intraperitoneal injection of 25–30 mg/kg of pentobarbital sodium. At the time of sacrifice, serum specimens were collected from all of the rats, and the right femur and right tibia were isolated.

The serum samples were stored at −20°C. The urine and serum samples were used for the measurements of the biochemical markers as described below. The femurs were stored at −20°C and then used for biomechanical testing as described below. The tibiae were processed for bone histomorphometric analyses. The bones were fixed in 40% cold ethanol overnight, and then cut into three parts using an Isomet saw (Buehler, Lake Bluff, IL, USA). The proximal tibial metaphysis and tibial diaphysis with the fibular junction were stained with Villanueva Osteochrome Bone Stain (Polyscience, Warrington, PA, USA) for 5 d. The specimens were 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 according to the method of Erben (10). Cross-sections of the tibial diaphysis just proximal to the tibio-fibular junction were cut at 40-μm thickness using a diamond wire Histo-Saw machine (Delaware Diamond Knives, Wilmington, DE, USA), 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 proximal tibial metaphysis were cut at 8-μm or 4-μm thickness using a microtome (Leica RM2155; Leica Inc., Nussloch, Germany). The 8-μm sections were then transferred onto chromium-gelatin-coated slides and dried overnight under a press at 42°C. All the sections were coverslipped with Eukitt (Calibrated Instruments, Hawthorne, NY, USA) for the static and dynamic histomorphometric analyses. For tartrate-resistant acid phosphatase (TRAP) histochemistry, 8-μm sections of the proximal tibial metaphysis were deplasticized with three changes of 2-methoxyethylacetate for 30 min each, two changes of acetone for 5 min each, and sequential changes of ethanol (95%, 70%, and 40%), followed by two changes of deionized water for 5 min each for rehydration. The deplasticized and rehydrated sections (8-μm thickness) were placed in 0.1 M acetate buffer at pH 5.0 for 5 min, and the TRAP reaction was performed using a leukocyte acid phosphatase kit (Sigma Chemical). Sections stained for TRAP were counterstained with Mayer’s hematoxylin (1 min) and then air-dried and mounted with a plastic UV mounting medium (Polysciences Inc., Warrington, PA, USA). For Goldner Trichrom staining to measure the Ob/BS, adjacent 4-μm sections of the proximal tibial metaphysis were deplasticized and rehydrated, followed by the procedure for Goldner Trichrom staining, and mounted with Eukitt (Calibrated Instruments, Hawthorne, NY, USA).
Urine and serum biochemical analyses. Urinary deoxypyridinoline (DPD) levels were measured by enzyme-immunoassay (EIA) using a Pyrilinks-D kit (Metra Biosystems Inc., CA, USA). Serum calcium and phosphorus levels were measured in automated equipment (Dada Behring Model RXl, Bakersfield, CA, USA). Serum osteocalcin (OC) levels were measured by immunoradiometric assay (IRMA) using a Rat Osteocalcin IRMA kit (Immutopics, Inc., CA, USA).

Biomechanical testing. The mechanical properties of the diaphysis of the femurs were evaluated by the three-point bending test. Load was applied midway between two supports placed 15 mm apart on the bone. The femur was positioned so that the loading point was at the center of the femoral 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 for 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 the maximum load, stiffness, and breaking energy.

Just after the three-point bending test of the femoral diaphysis, the distal metaphysis was isolated over a length of 10 mm from the joint surface of the femoral condyle. The mechanical properties of this segment were then measured by the compression test. Compressive load was applied on the specimens from the lateral 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 for 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 the maximum load, stiffness, and breaking energy.

Bone histomorphometry of the tibia. A digitizing morphometry system was used to measure the bone histomorphometric parameters of the tibial specimens. The system consisted of an epifluorescence microscope (Nikon E-400, Osteometrics, Atlanta, GA, USA), an Osteo Measure High Resolution Color Subsystem (Osteometrics), and a digitizing pad (Numonics 2206; Numonics Corp., Montomerville, PA, USA) coupled to an IBM computer, and a morphometry program (Osteometrics). The measured parameters for cancellous bone included the total tissue volume (TV), bone volume (BV), bone surface (BS), eroded surface (ES), single- and double-labeled surfaces (sLS and dLS, respectively), and osteoblast surface (ObS). These data were used to calculate the cancellous bone volume (BV/TV), trabecular number (Tb N), trabecular thickness (Tb Th), trabecular separation (Tb Sp), ES/BS, MS/BS [(sLS−2×dLS)/BS], mineral apposition rate (MAR), bone formation rate (BFR)/BS, BFR/BV, and ObS/BS, in accordance with the standard nomenclature described by Parfitt et al. (11).

In the present study, the region of cancellous bone measured was 1–4 mm distal to the lower margin of the growth plate in the proximal tibia, which consists of secondary spongiosa. Cells showing positive staining for TRAP were counted in the region extending from the distal end of the growth plate to a distance of 0.2 mm from the growth plate, and the number of osteoclasts (N Oc) and the osteoclast surface (OcS) per BS were calculated. The measured parameters for cortical bone were the total tissue area (Tt Ar), marrow area (Ma Ar), endocortical ES, peristeal and endocortical BS, sLS, dLS, and the interlabel width. These data were used to calculate the cortical bone area (Ct Ar), endocortical ES/BS, and peristeal and endocortical MS/BS [(sLS−2×dLS)/BS], MAR, and BFR/BS.

Statistical analysis. All the data were expressed as means ± standard deviation (SD). Multiple comparisons of data among the groups were performed by analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD) test. All 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

Changes in body weight

Table 1 shows that the initial body weight did not differ significantly among the four experimental groups. The body weight more markedly increased 6 wk after surgery in ovariectomized rats than in sham-operated rats. However, the body weight tended to decrease from 6 wk to 12 wk after surgery in the all groups, probably due to the influence of dosing.

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Baseline</th>
<th>6 wk (start of treatment)</th>
<th>12 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>366 ± 32</td>
<td>381 ± 48</td>
<td>351 ± 38</td>
</tr>
<tr>
<td>OVX</td>
<td>364 ± 29</td>
<td>446 ± 23◊</td>
<td>404 ± 9◊</td>
</tr>
<tr>
<td>Vehicle</td>
<td>367 ± 42</td>
<td>409 ± 57</td>
<td>390 ± 33◊</td>
</tr>
<tr>
<td>Alendronate</td>
<td>371 ± 30</td>
<td>436 ± 24◊</td>
<td>388 ± 30◊</td>
</tr>
<tr>
<td>Alfacalcidol</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. ANOVA with Fisher’s PLSD test was used to compare the data among the groups.

◊ Significant vs Sham.
Table 2. Biochemical markers.

<table>
<thead>
<tr>
<th></th>
<th>6 wk (start of treatment)</th>
<th>9 wk</th>
<th>12 wk</th>
<th>Osteocalcin (ng/ml)</th>
<th>Calcium (mg/dL)</th>
<th>Phosphorus (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham O VX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>44.3±10.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.3±18.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.5±20.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.3±5.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.9±0.3</td>
<td>6.7±0.3</td>
</tr>
<tr>
<td>Alendronate</td>
<td>48.3±18.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.7±9.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.4±2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.9±2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.2±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.0±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alfacalcidol</td>
<td>49.8±13.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>38.7±16.9&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>24.8±10.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.7±12.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>10.1±0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.9±0.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. ANOVA with Fisher’s PLSD test was used to compare the data among the groups.
<sup>a</sup> Significant vs Sham.
<sup>b</sup> Significant vs Vehicle.
<sup>c</sup> Significant vs Alendronate.

Fig. 1. Bone histomorphometric analysis of the cancellous bone of the proximal tibial metaphysis—Structural parameters.—Data are expressed as mean±SD. ANOVA with Fisher’s PLSD test was used to compare the data among the groups. a: significant vs Sham, b: significant vs Vehicle, c: significant vs Alendronate. BV/TV: bone volume/total tissue volume, Tb N: trabecular number, Tb Th: trabecular thickness, Tb Sp: trabecular separation.

Table 3. Histomorphometric analysis of the cancellous bone of the proximal tibial metaphysis—Formative and resorptive variables.—

<table>
<thead>
<tr>
<th></th>
<th>ES/BS(%)</th>
<th>N.Oc/BS (#/mm)</th>
<th>OcS/BS (%)</th>
<th>Obs/BS (%)</th>
<th>MS/BS (%)</th>
<th>MAR (µm/d)</th>
<th>BFR/BS (µm³/µm²/d)</th>
<th>BFR/BV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham O VX</td>
<td>7.2±3.4</td>
<td>1.11±0.20</td>
<td>4.2±0.6</td>
<td>13.4±0.5</td>
<td>10.6±4.5</td>
<td>0.96±0.17</td>
<td>9.9±4.3</td>
<td>119±50</td>
</tr>
<tr>
<td>Vehicle</td>
<td>12.6±4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.40±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.4±2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.6±2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.2±3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.88±0.12</td>
<td>17.2±5.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>281±63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alendronate</td>
<td>8.6±1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.61±0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.3±1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.3±2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.9±2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.62±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.5±1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77±18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alfacalcidol</td>
<td>7.6±3.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.14±0.45&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.9±1.8&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>18.8±2.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>22.4±4.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.86±0.26&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>19.1±5.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>213±53&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. ANOVA with Fisher’s PLSD test was used to compare the data among the groups.
<sup>a</sup> Significant vs Sham.
<sup>b</sup> Significant vs Vehicle.
<sup>c</sup> Significant vs Alendronate.
ES: eroded surface, BS: bone surface, N.Oc: number of osteoclasts, Obs: osteoblast surface, MS: mineralizing surface, MAR: mineral apposition rate, BFR: bone formation rate, BV: bone volume.
levels in the ovariectomized osteopenic rats and increased the serum phosphorus level, without inducing any significant hypercalcemia.

*Bone histomorphometric analysis of the cancellous bone of the proximal tibial metaphysis*

The cancellous BV/TV, Tb N, and Tb Th were decreased, and the Tb Sp was increased, 12 wk after OVX (Fig. 1), as a result of increased bone resorption (ES/BS, N.Oc/BS, OcS/BS) and bone formation (ObS/BS, MS/BS, BFR/BS, BFR/BV) (Table 3). Alendronate increased the BV/TV, Tb N, and Tb Th and decreased the Tb Sp in the ovariectomized osteopenic rats relative to the values observed in the OVX-Vehicle group, but the values were not restored to those observed in the Sham group (Fig. 1). Similarly, alfalcacidol increased the BV/TV, Tb N, and Tb Th and decreased the Tb Sp in the ovariectomized osteopenic rats relative to the values observed in the OVX-Vehicle group, but the values were not restored to those observed in the Sham group except for Tb Th (Fig. 1). The increases in the BV/TV and Tb Th induced by alfalcacidol were more pronounced than those induced by alendronate.

The alterations of the structural parameters induced by alendronate were attributable to the prevention of the increases in bone resorption (ES/BS, N.Oc/BS, OcS/BS) and bone formation (ObS/BS, MS/BS, BFR/BS, BFR/BV) by the drug (Table 3). In particular, the MAR in the alendronate group was lower than that in the Sham group (Table 3). On the other hand, the alterations of the structural parameters induced by alfalcacidol were attributable to decreased bone resorption (ES/BS, N.Oc/BS, OcS/BS), while bone formation (ObS/BS, MS/BS, BFR/BS) was maintained (Table 3). The suppression of bone resorption (OcS/BS) induced by alfalcacidol was less pronounced than that induced by alendronate.

*Bone histomorphometric analysis of the cortical bone of the tibial diaphysis*

OVX increased the Tt Ar and Ma Ar (Fig. 2) by

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**Table 4. Histomorphometric analysis of the cortical bone of the tibial diaphysis—Formative and resorptive variables—**

<table>
<thead>
<tr>
<th>Periosteal</th>
<th>Endocortical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>MS/BS (%)</td>
<td>MAR (μm/d)</td>
</tr>
<tr>
<td>Sham OVX</td>
<td>39.6±10.7</td>
</tr>
<tr>
<td>Vehicle</td>
<td>66.4±10.2a</td>
</tr>
<tr>
<td>Alendronate</td>
<td>51.8±20.0b</td>
</tr>
<tr>
<td>Alfalcacidol</td>
<td>53.8±20.0b</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. ANOVA with Fisher's PLSD test was used to compare the data among the groups.

a Significant vs Sham.

b Significant vs Vehicle.

c Significant vs Alendronate.

increasing periosteal bone formation (MS/BS, BFR/BS) and endocortical bone resorption (ES/BS) and, subsequently, increased endocortical bone formation (MS/BS, MAR) (Table 4), without affecting the Ct Ar (Fig. 2). Alendronate prevented the increase in the Ma Ar, restoring it to the value observed in the Sham group (Fig. 2). Alfalcacidol also prevented the increase of the Ma Ar and restored it to the value observed in the Sham group; the values of the Tt Ar and Ct Ar remained higher in the alfalcacidol group than in the Sham group (Fig. 2). Furthermore, alfalcacidol also decreased endocortical bone resorption (ES/BS), while maintaining or even stimulating endocortical bone formation (MS/BS, MAR, BFR/BS) (Table 4).

Biomechanical test of the femur

Figure 3 shows that OVX was associated with a decrease of the maximum load of the femoral distal metaphysis, without any change in the mechanical properties of the femoral diaphysis. Alendronate had no effect on the mechanical properties of either the femoral distal metaphysis or the femoral diaphysis. On the other hand, alfalcacidol prevented the loss of the maximum load and increased the stiffness and breaking energy of the femoral distal metaphysis, without having any effect on the mechanical properties of the femoral diaphysis; the maximum load of the femoral distal metaphysis was restored to the value observed in the Sham group.

DISCUSSION

The present study demonstrated that alendronate and alfalcacidol increased the cancellous BV/TV in the ovariectomized osteopenic rats relative to the values observed in the OVX-Vehicle group. The increase in the cancellous BV/TV induced by alfalcacidol was more pronounced than that induced by alendronate. Furthermore, alfalcacidol, but not alendronate, increased the Ct Ar, and prevented the loss of the maximum load of the femoral distal metaphysis induced by ovariectomy. Thus, the present study clearly showed that alendronate and alfalcacidol had differential therapeutic effects on the cancellous and cortical bone mass and mechanical properties in ovariectomized osteopenic rats.

OVX was associated with decreases of the cancellous BV/TV, Tb N, and Tb Th and an increase of the Tb Sp of the proximal tibial metaphysis, as a result of increased bone turnover, and a decrease of the maximum load of the femoral distal metaphysis. OVX was also followed by increases of the Tt Ar and Ma Ar as a result of increased periosteal bone formation and endocortical bone resorption, without any change in the Ct Ar or mechanical properties of the femoral diaphysis. Therefore, the pharmacological efficacy of alendronate and alfalcacidol in ovariectomized osteopenic rats, in terms of their beneficial effects on the cancellous bone, and also on periosteal bone formation and endocortical
bone resorption (1,2), can certainly be translated into clinical efficacy in postmenopausal women with osteoporosis. In a previous study, we confirmed that OVX resulted in cancellous osteopenia by 6 wk after the surgery in 6-mo-old rats, without any associated cortical osteopenia (13). Based on this report, we assumed that our study animals would also have developed cancellous osteopenia by 6 wk after the OVX.

Alendronate increased the BV/TV, Tb N, and Tb Th, and decreased the Tb Sp in the ovariectomized osteopenic rats relative to the values observed in the OVX-Vehicle group, by adequately suppressing bone turnover. Thus, alendronate improved not only the connectivity of trabecular bone, but also its thickness in ovariectomized osteopenic rats; nevertheless, the cancellous BV/TV was not completely restored to the value observed in the Sham group. This may reflect the limitation of alendronate in increasing the cancellous bone mass in ovariectomized osteopenic rats, because the increase in the cancellous bone mass depends on the amount of remodeling space that can be filled with trabecular bone by the anti-resorptive effect of the drug.

Alfacalcidol increased the BV/TV, Tb N, and Tb Th and decreased the Tb Sp in the ovariectomized osteopenic rats relative to the values observed in the OVX-Vehicle group, by suppressing bone resorption, while maintaining bone formation. These effects have rarely been reported in ovariectomized osteopenic rats. Thus, like alendronate, alfacalcidol also improved the connectivity of the trabecular bone as well as its thickness in ovariectomized osteopenic rats. However, the suppression of bone resorption induced by alfacalcidol was milder than that induced by alendronate, and alfacalcidol, unlike alendronate, also maintained bone formation. Thus, while alfacalcidol had a milder anti-resorptive effect than alendronate on cancellous bone in ovariectomized osteopenic rats, it appeared to have the potential to maintain bone formation, differing in this respect from alendronate. The increase in the Tb Th induced by alfacalcidol was more apparent than that induced by alendronate, probably due to maintained bone formation; however, alfacalcidol also did not completely restore the cancellous BV/TV to the value observed in the Sham group. Because no apparent hypercalcemia was induced by alfacalcidol in our dose setting, treatment with higher-doses of alfacalcidol will probably induce a further increase of the cancellous BV/TV in ovariectomized osteopenic rats.

Alfacalcidol prevented the loss of the maximum load of the femoral distal metaphysis, whereas despite increasing the cancellous BV/TV, alendronate had no effect on the mechanical properties of the femoral distal metaphysis. The more marked effect of alfacalcidol than alendronate on the mechanical properties of the femoral distal metaphysis, which is rich in cancellous bone, may be related to the effects of alfacalcidol on the trabecular structure, bone turnover (resorption), and bone mineralization (bone formation) (14, 15). Thus, firstly, alfacalcidol induced a more marked increase in the cancellous BV/TV and Tb Th than alendronate, suggesting its superior beneficial effect on the trabecular structure.

Secondly, bone resorption was suppressed more markedly by alendronate than by alfacalcidol. Thirdly, alfacalcidol, unlike alendronate, maintained bone formation, in terms of mineralization. Thus, the greater improvement of the cancellous bone structure, milder suppression of bone resorption, and maintained mineralization following alfacalcidol treatment as compared to that following alendronate treatment might explain the superior efficacy of alfacalcidol in improving the mechanical properties of skeletal sites rich in cancellous bone.

The MAR and serum OC level were lower in the alendronate group than in the Sham group, presumably on account of the marked suppression of bone formation by alendronate. Sato et al. (14) reported that long-term treatment with alendronate attenuated cancellous BV/TV loss, but had little effect on the mechanical properties of the lumbar vertebrae in ovariectomized rats. This could be attributable to the marked suppression of bone formation by alendronate adversely affecting the bone quality in ovariectomized rats. Thus, in the present study also, the nonsignificant effect of alendronate on the mechanical properties of the femoral distal metaphysis might be attributable, at least in part, to the marked suppression of bone formation.

Both alendronate and alfacalcidol similarly prevented the OVX-induced increase in the Mn Ar by suppressing endocortical bone resorption. However, only alfacalcidol maintained the Tt Ar around the value observed in the OVX-Vehicle group and increased the Ct Ar to beyond the value observed in the Sham group. On the other hand, alendronate, but not alfacalcidol, influenced the OVX-induced periosteal cortical expansion (as measure by the Tt Ar), by causing a more marked suppression of periosteal bone formation. However, neither alendronate nor alfacalcidol had any effect on the mechanical properties of the femoral diaphysis; thus, other factors than the cortical bone mass, periosteal bone formation, and endocortical bone formation and resorption might contribute to the mechanical properties of cortical bone.

Clinically, randomized controlled trials have demonstrated that alendronate at 5 mg/d is more effective than alfacalcidol at 1 µg/d in increasing the lumbar BMD and reducing the incidence of vertebral fractures in Japanese postmenopausal women with osteoporosis (1, 2). Nevertheless, in the present study, alfacalcidol at 0.5 µg/kg induced a greater increase of the cancellous BV/TV than alendronate at 2.5 mg/kg, and in addition, unlike alendronate, alfacalcidol also increased the maximum load of the femoral distal metaphysis. It appeared that alendronate was well absorbed through the stomach of the rats in the present study, because a significant reduction in bone turnover was observed. This discrepancy between the clinical and experimental results may be due to the differential responses of cancellous bone to alfacalcidol between ovariectomized rats and postmenopausal women (humans). In fact, the potent preventive effect of alfacalcidol on cancellous bone loss
after OVX in rats has been confirmed in previous studies (7, 8); thus, alfacalcidol might exert greater beneficial effects on rat bones than on human bones.

In conclusion, the present study demonstrated that both alendronate and alfacalcidol increased the cancellous bone mass by suppressing bone turnover in ovarieectomized osteopenic rats. However, the effect of alfacalcidol on cancellous bone mass was more pronounced than that of alendronate. In addition, unlike alendronate, alfacalcidol also increased the cortical bone mass, and prevented the loss of the maximum load of the femoral distal metaphysis in ovarieectomized osteopenic rats. Thus, the present study clearly showed that alendronate and alfacalcidol had differential therapeutic effects on the cancellous and cortical bone in ovarieectomized osteopenic rats.

REFERENCES


