Effects of Continuous Alendronate Treatment on Bone Mass and Mechanical Properties in Ovariectomized Rats: Comparison with Pamidronate and Etidronate in Growing Rats

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ABSTRACT
Alendronate is a potent inhibitor of bone resorption. To investigate the relationship between antiresorptive activity and bone-related side effects, we studied the effect of 2 months of daily alendronate (0.04, 0.2, 1.0 or 5.0 mg/kg/day) treatment on the strength of the femoral shaft and neck and on the bone mass of ovariectomized rats. The p.o. administration regimen began immediately after ovariectomy at 6 weeks of age, and the results were compared with pamidronate (0.2, 1.0 or 5.0 mg/kg/day) or etidronate (5.0, 25.0 or 125.0 mg/kg/day) treatment. In the femoral epiphysis and neck, a preventive effect of alendronate on loss of bone mineral density was observed at the dose of 1.0 mg/kg. The alendronate-treated group did not show significant alteration of the breaking load or the cross-sectional shape of the femoral midshaft. Similar results were obtained in the femoral neck strength and femoral neck geometry. In histomorphometric analysis of tibial metaphyses, alendronate inhibited the ratio of osteoid volume to tissue volume and the mineral apposition rate at a dose of 0.2 mg/kg compared with the ovariectomized control. In contrast, etidronate tended to increase osteoid volume/bone volume at 125 mg/kg. From these results, we conclude that p.o. alendronate-treatment prevented the decrease in bone mineral density and maintained the mechanical properties of bone after ovariectomy without impairing bone mineralization in growing rats.

Bisphosphonates are potent inhibitors of bone resorption and have been shown to be effective in preventing immobilization or estrogen deficiency-induced osteopenia both in experimental animal models and in clinical trials (Thompson et al., 1990; Seedor et al., 1991; Chestnut et al., 1995 and Tucci et al., 1996). Schenk et al. (1986) histomorphometrically compared the effect of bisphosphonates on metaphyseal modeling or remodeling in growing rats. They showed that alendronate powerfully inhibited bone resorption without affecting mineralization. In a later paper, Shinoda et al. (1983) reported that etidronate impaired mineralization at the same dose that exhibited the maximal antiresorptive effect. In contrast, impairment of mineralization by pamidronate was observed only at a dose 10 times higher than that which had maximal preventive effect on resorption (Shinoda et al., 1983). Because alendronate does not impair bone mineralization at doses that maximally inhibit bone resorption (Rodan et al., 1993), it should not be associated with mineralization defects or osteomalacia, effects that have been seen with nonselective bisphosphonates (Heaney and Saville, 1976).

The mineralization defect and bone growth retardation seen in connection with nonselective bisphosphonates led us to become concerned about the effects of such treatment on the final determinant, i.e., bone quality. For osteoporosis treatment, the mechanical strength of bones is the final determinant of the usefulness of the therapy. Previously, attention has been paid to bone biomechanical aspects after prolonged periods of bisphosphonate treatment. Alendronate preserved the mechanical properties of vertebrae and femur in ovariectomized rats (Toolan et al., 1992) and in ovariectomized baboons (Balena et al., 1993). Furthermore, daily treatment with alendronate reduced the incidence of vertebral fractures (Liberman et al., 1995; Black et al., 1996). However, although the effects of etidronate in preserving bone mass and reducing the risk of vertebral fractures were favorable (Harris et al., 1993), the requirement for intermittent dosing to minimize the possibility of osteomalacia (Khari et al., 1974) limits its general acceptance in the treatment of osteoporosis. Thus the effects of these drugs on bones are strongly dependent on the specific chemical structure of each bisphosphonate (Fleisch, 1987), and their safety and effect on bone structure and quality must be investigated. Defective mineralization and reduced longitudinal bone growth may interfere with bone quality and structure as a result of the

ABBREVIATIONS: OVX, ovariectomy; BMD, bone mineral density; BMC, bone mineral content; BV/TV, bone volume/tissue volume; ES/BS, erosion surface/bone surface; OV/BV, osteoid volume/bone volume; MAR, mineral apposition rate; LBG, longitudinal bone growth; DEXA, dual-energy X-ray absorptiometry.
use of nonselective bisphosphonates. In any effort to estimate the relationship between the antiresorptive activity of bisphosphonates and defective mineralization or reduced bone growth, growing rats are more suitable than aged rats.

In this study, we focused on the effect of three bisphosphonates (alendronate, pamidronate and etidronate) on bone quality in growing rats. To investigate the relationship between the antiresorptive activity and bone-related side effects, we studied the effect of 2 months of alendronate, pamidronate and etidronate treatment, with the p.o. administration begun after the ovariectomy, on the strength of the femoral shaft and neck and on bone mass. Furthermore, to evaluate the effect of alendronate and etidronate on tibial metaphyseal bone modeling or remodeling, we conducted static and dynamic histomorphometric analyses. We also examined the geometric parameters of the femur and femoral neck to determine whether 2 months of daily administration of bisphosphonates would alter the architecture or geometry of bone in growing rats.

Materials and Methods

Compounds. Alendronate, pamidronate and etidronate were synthesized at the Teijin Institute for Bio-Medical Research (Tokyo, Japan). Tetracycline was purchased from Pfizer Pharmaceuticals Co. (Tokyo, Japan), and calcine was commercially obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were at least reagent grade, or pure.

Study design. Animals. Sprague-Dawley female rats (5 weeks old), obtained from Charles River Japan Ltd. (Tokyo, Japan), were used. All rats were housed individually at 24 ± 2°C at a relative humidity of 55 ± 10% with a 12-h:12-h light-dark cycle. The rats were fed standard rat chow (CE2) obtained from Clea Japan Inc. (Tokyo, Japan) and were given water ad libitum.

At 6 weeks of age, the animals were subjected to bilateral OVX or a sham operation (Sham) under anesthesia induced by i.p. injection with sodium pentobarbital (50 mg/kg/ml, Dinabot Inc., Osaka, Japan). The rats were divided into 12 groups. Sham and OVX groups were orally administered pure vehicle (phosphate buffered saline: PBS) adjusted to pH 7.4 in a volume of 1.0 ml/kg. Four groups were orally administered 0.04, 0.2, 1.0 and 5.0 mg/kg of alendronate. Three groups were orally administered pamidronate at 0.2, 1.0 and 5.0 mg/kg, respectively. The remaining three groups were orally administered etidronate at 5.0, 25 and 125 mg/kg, respectively. The dosage levels of alendronate were determined on the basis of the s.c. dose at which the pharmacological effect appears in ovariectomized rats (Seedor et al., 1991) and the oral absorption rate in rats (Lin et al., 1991). On the basis of reports of studies comparing the effects of bisphosphonates on bone mass of the tibia epiphysis in growing rats, the maximal antiresorptive effect of alendronate was seen at a dose 10 times lower than that for the maximal effect of pamidronate and at a dose 100 times lower than that for maximal etidronate effect (Schenk et al., 1986; Shinoda et al., 1983). No large differences in pharmacokinetics were found among these three bisphosphonates (Lin et al., 1991; Wingen and Schmahl, 1987; Michael et al., 1972; Lin, 1996). In accordance with these results, the dose levels of etidronate were set 25 times higher than those of alendronate. With pamidronate, the dosage levels were set 5 times higher than those of alendronate. Administration volume of bisphosphonates or vehicle in orally treated rats was 1.0 ml/kg. The bisphosphonates were administered p.o. once a day for 2 months after the operation. For dynamic histomorphometry, animals were treated i.p. with tetracycline (25 mg/kg) 7 days before sacrifice and s.c. with calcine (5 mg/kg) 1 day before sacrifice. At the end of an experiment, rats were anesthetized with ether, and blood was obtained from the abdominal aorta.

Plasma was collected after centrifugation at 3000 rpm for 10 min. The femurs and tibiae were removed immediately.

Plasma biochemistry. Plasma calcium (Ca), inorganic phosphorus (P) and alkaline phosphatase activity (ALPase) were determined after sacrifice at the end of the experiment. The concentrations of Ca, P, and ALPase in plasma were measured with an autoanalyzer (model 736-20, Hitachi Co. Ltd., Tokyo, Japan) using Clinimate Ca, Clinimate IP, and Clinimate ALP (Daichi Pure Chemicals Co. Ltd., Tokyo, Japan).

Measurement of dry bone weight, bone volume and ash weight. After removal of the soft tissue, the left tibia and femur were dehydrated with ethanol, and fat was removed with diethyl ether. After the bones were allowed to air-dry, the dry bone weight and bone volume were measured with a plethysmometer (model TK-101, Unicom Co. Ltd., Chiba, Japan). Ash weight was measured after ashing the bone at 800°C in an asher (Tokyo Rikakikai Co. Ltd., Tokyo, Japan). The ash components were then dissolved in 6 N HCl and measured for Ca and P, content with the autoanalyzer mentioned above.

Bone densitometry. The right femurs were cleaned of soft tissue and scanned with a DEXA (Hologic QDR-2000, Waltham, MA) equipped with Regional High-Resolution Scan software. The scan field size was 3.48 × 1.41 cm, and the resolution was 0.0254 × 0.0127 cm. The longitudinal size of the femur was measured on the scan field with the software, and the field was adjusted to accommodate the whole femur. The field of the whole femur was divided into four equal fields, R1 to R4. The femoral scan images were obtained, and bone area, BMC and BMD of the whole femur and of distal femoral epiphysis (R1), femoral shaft (R2 and R3) and proximal femur (R4) were determined, as was the proximal end of the femur (R5), which contains the femoral head, femoral neck and femoral greater trochanter (fig. 1). The stability of the instrument was calibrated before each measurement was made. The coefficients of variation for the paired measurement of the BMC and BMD of standard samples by this technique were 0.8% and 1.0%, respectively.

Bone biomechanics. Three-point bending. Biomechanical testing was conducted with a Bone Strength Measuring Apparatus (Model MZ-500D; Maruto Testing Machine Co. Ltd., Tokyo, Japan). Data were recorded and analyzed with the software package “Chutaro” on a computer (Maruto Testing Machine Co. Ltd., Tokyo, Japan). The three-point bending test was performed as previously described (Mølster, 1984). Specifically, femurs were positioned so
that one fulcrum was at the distal 1/6 part of the total length and the other was at the proximal 1/6 part of it. The breaking force was applied perpendicularly to the long axis of the bone at the speed of 6 mm/min. The femurs were broken from the anterior to the posterior plane. The breaking load (newtons) and displacement (millimeters) at failure were recorded. The total cross-sectional area (square millimeters) of each diaphyseal specimen was calculated from the outer anteroposterior (AP) diameter (millimeters) and right-left (RL) diameter (millimeters). The marrow area (square millimeters) was also calculated from the inner AP and RL diameters (millimeters) by a digital micrometer (Mitsutoyo Ltd., Tokyo, Japan). The cortical bone area (square millimeters) was determined from the total cross-sectional area and the marrow area.

Femoral neck-compression. The axial compression strength of the femoral neck region was tested by loading the femoral neck parallel to the shaft of the femur. The specially constructed fixation device holding the specimen was then placed in the testing machine. After the three-point bending test, the left proximal femur was used. The distal side of specimen was embedded in methacrylate-resin (OSTRON-II, GC Dental Products Co., Aichi, Japan) and fixed to the fixation device. A vertical load conducted by a flat-surfaced brass cylinder was applied to the axis of the diaphysis and moved at a constant rate of 1 mm/min until fracture of the femoral neck occurred. During compression, load-deformation curves were recorded continually by a computerized monitor linked to the tester. The ultimate strength (newtons) was obtained directly from the load-deformation curves at the level of maximal load. The structural stiffness (newtons per millimeter) was determined by the slope of the curve. Femoral neck geometry was measured as previously described (Sogaad et al., 1994). On each X-ray image of the mounted femur specimen, the angle (θ) was determined, and the corresponding cervical-diaphyseal angle (θ) was calculated as 90° – α. To determine the length of the femoral neck, the distance from the junction of the neck to the neck-head was measured. The total cross-sectional area (square millimeters) of femoral neck specimen was calculated from the outer anteroposterior (AP) diameter (millimeters) and the right-left (RL) diameter (millimeters).

Bone histomorphometry. Histomorphometry of the right tibia was carried out in Sham and OVX groups and in the groups treated with alendronate or etidronate. Right tibia were fixed in 70% ethanol, dehydrated through graded concentrations of ethanol up to 100% and cleared in xylene. After fixation, a low-speed diamond wheel saw (Mitsutoyo Ltd., Tokyo, Japan) and embedded in methyl methacrylate resin (MMA, Polysciences Inc., War-ington, PA) and stained with Villanueva Osteochrome (Polysciences Inc., War-ington, PA) and embedded in methyl methacrylate resins (MMA, Polysciences Inc.). Frontal sections of proximal tibial metaphyses of 200 μm in thickness were cut with the low-speed diamond wheel saw and then ground to 30 μm for histomorphometric analysis (Exakt Apparatebau GmbH, Germany). Static and dynamic histomorphometry of tibial metaphyses was performed under a microscope using an image-analyzing software package (Osteoplan-II: Kontron Co., To-kyo, Japan). The OV/BV, ES/BS and trabecular BV/TV ratios, as well as the MAR (μm/day) and longitudinal bone growth (μm/day), were measured.

Statistical analysis. All data were expressed as the mean ± S.D. except those for histomorphometric parameters, which were pre-sented as the mean ± S.E.M. The significance of differences between the OVX group and other groups was evaluated by a post-hoc Dunnett’s two-tailed test. Linear regression analysis was used to corre-late BMD with bone strength measurements.

Results

Body weight and plasma biochemical parameters. The final body weights of animals in the OVX group (335 ± 22 g) increased significantly relative to those of the Sham group (260 ± 25 g, P < .05 vs. OVX). At no dose level did alendronate have any significant effect on this weight gain observed for the OVX group, nor did pamidronate or etidr-onate. Plasma Ca concentration was significantly lower in the OVX group (9.9 ± 0.1 mg/dl) than in the Sham group (10.2 ± 0.3 mg/dl, P < .05 vs. OVX). We observed no significant differences in plasma Ca concentration between the OVX and bisphosphonate groups. There were no significant differences in plasma P<sub>i</sub> concentration between any two groups. Plasma ALPase values were significantly higher in the OVX group (351 ± 78 U/l) than in the Sham group (251 ± 82 U/l, P < .05 vs. OVX). None of the bisphosphonates signifi-cantly affected plasma ALPase values at any dose level.

Dry bone weight, bone volume and ash weight. Table 1 summarizes the effects of OVX and treatment of ovariec-tomized rats with bisphosphonates on dry bone weight, bone volume, ash weight, bone Ca content, the dry bone weight per bone volume, the bone Ca content per bone volume and the Ca/P<sub>i</sub> ratio of the right tibia.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Dry Weight (mg)</th>
<th>Volume (ml)</th>
<th>Ash Weight (mg)</th>
<th>Ca (mg)</th>
<th>Ca/P&lt;sub&gt;i&lt;/sub&gt;</th>
<th>Dry Wt./Vol. (g/ml)</th>
<th>Ca/Vol. (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>12</td>
<td>389.6 ± 27.6</td>
<td>0.31 ± 0.03*</td>
<td>238.1 ± 16.2</td>
<td>78.3 ± 5.2</td>
<td>1.88</td>
<td>1.24 ± 0.04*</td>
<td>201.1 ± 5.8</td>
</tr>
<tr>
<td>OVX</td>
<td>12</td>
<td>404.0 ± 20.9</td>
<td>0.35 ± 0.02</td>
<td>244.6 ± 12.1</td>
<td>79.8 ± 3.9</td>
<td>1.92</td>
<td>1.16 ± 0.04</td>
<td>197.7 ± 4.1</td>
</tr>
<tr>
<td>Alendronate 0.04 mg/kg</td>
<td>8</td>
<td>417.5 ± 36.7</td>
<td>0.35 ± 0.03</td>
<td>256.7 ± 16.2</td>
<td>91.4 ± 6.6</td>
<td>1.99</td>
<td>1.18 ± 0.03</td>
<td>219.3 ± 6.3*</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>8</td>
<td>416.4 ± 21.8</td>
<td>0.35 ± 0.02</td>
<td>256.2 ± 12.2</td>
<td>91.7 ± 4.5*</td>
<td>1.98</td>
<td>1.21 ± 0.03</td>
<td>220.3 ± 5.0*</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>7</td>
<td>446.4 ± 32.1</td>
<td>0.36 ± 0.02</td>
<td>272.0 ± 23.6</td>
<td>98.1 ± 7.1*</td>
<td>2.00</td>
<td>1.23 ± 0.02</td>
<td>219.7 ± 7.2*</td>
</tr>
<tr>
<td>5.0 mg/kg</td>
<td>7</td>
<td>478.3 ± 37.3*</td>
<td>0.37 ± 0.02</td>
<td>298.3 ± 24.8*</td>
<td>106.9 ± 8.7</td>
<td>2.03</td>
<td>1.30 ± 0.05*</td>
<td>233.4 ± 4.4*</td>
</tr>
<tr>
<td>Pamidronate 0.2 mg/kg</td>
<td>8</td>
<td>411.7 ± 35.7</td>
<td>0.36 ± 0.03</td>
<td>292.5 ± 107.8*</td>
<td>91.0 ± 7.2</td>
<td>1.97</td>
<td>1.16 ± 0.03</td>
<td>221.2 ± 3.1*</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>7</td>
<td>416.2 ± 48.7</td>
<td>0.36 ± 0.04</td>
<td>254.0 ± 29.5</td>
<td>94.8 ± 9.3</td>
<td>1.98</td>
<td>1.18 ± 0.05</td>
<td>231.1 ± 39.1*</td>
</tr>
<tr>
<td>5.0 mg/kg</td>
<td>8</td>
<td>416.6 ± 35.5</td>
<td>0.34 ± 0.03</td>
<td>262.0 ± 23.8</td>
<td>92.2 ± 7.3</td>
<td>1.97</td>
<td>1.24 ± 0.05</td>
<td>221.0 ± 9.4*</td>
</tr>
<tr>
<td>Etidronate 5.0 mg/kg</td>
<td>8</td>
<td>402.6 ± 36.8</td>
<td>0.35 ± 0.03</td>
<td>246.0 ± 23.2</td>
<td>87.9 ± 7.1</td>
<td>1.98</td>
<td>1.17 ± 0.03</td>
<td>218.6 ± 3.3*</td>
</tr>
<tr>
<td>25.0 mg/kg</td>
<td>8</td>
<td>425.6 ± 27.8</td>
<td>0.36 ± 0.03</td>
<td>261.9 ± 16.3</td>
<td>91.8 ± 5.2</td>
<td>1.99</td>
<td>1.18 ± 0.03</td>
<td>215.9 ± 3.8*</td>
</tr>
<tr>
<td>125.0 mg/kg</td>
<td>8</td>
<td>440.8 ± 46.8</td>
<td>0.39 ± 0.03*</td>
<td>268.5 ± 31.2</td>
<td>91.8 ± 9.9</td>
<td>1.93</td>
<td>1.12 ± 0.04</td>
<td>208.3 ± 4.6*</td>
</tr>
</tbody>
</table>

Data are mean ± S.D. * P < .05 (vs. OVX group) by Dunnett’s two-tailed test.

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Significant differences were not observed in the alendronate and pamidronate groups relative to the OVX group at any dose level, but a significant increase (P < .05) vs. the OVX group in bone volume was observed in the etidronate (125.0 mg/kg) group.

With respect to bone mass, the OVX group exhibited significantly lower values for density of the tibia (dry bone weight per bone volume) than did the Sham group; this indicates that the bone mass density is decreased by ovariectomy. Values in all the bisphosphonate groups were higher than those in the OVX group, and values were significantly higher in the 5.0 mg/kg alendronate group (P < .05) vs. the OVX group. In fact, the value for bone mass density in the latter group exceeded that of the Sham group. For bone Ca content per tibial bone volume, alendronate treatment led to significantly higher values relative to the OVX group value, even at the low dose, 0.04 mg/kg. The pamidronate and etidronate groups also exhibited significantly higher values (P < .05) relative to the value for the OVX group at all doses.

Femoral BMD. The effects of OVX and subsequent treatment with bisphosphonates on the BMD of the right femur measured by DEXA are shown in figure 2, A–E. BMD values for the OVX group at R1 (distal epiphysis) and R5 (femoral neck and trochanter) were significantly lower than those for the Sham group, which indicates an OVX-elicited decrease in BMD in these areas. Alendronate at 1.0 and 5.0 mg/kg significantly inhibited the loss in BMD at R1 caused by OVX. Conversely, although pamidronate significantly inhibited the loss in BMD at 5.0 mg/kg, etidronate did not have any inhibitory effect at any dose level. There were no significant changes observed in R2 (mid-shaft: distal) and R3 (mid-shaft: proximal) BMD in the OVX group relative to the values for the Sham group. Alendronate at 5.0 mg/kg increased the BMD at R2. None of the bisphosphonates caused a significant change in BMD at R3. BMD values at R4 (proximal epiphysis) for the OVX group were lower than those for the Sham group, though not significantly so. The mean BMD value at R4 for the 5.0 mg/kg alendronate group was significantly higher than that for the OVX group.

Mechanical properties of femur. In the results obtained from the three-point bending test of the femoral shaft, alendronate and the other two bisphosphonates did not affect the breaking strength or deflection of the femoral shaft at any dose level tested. And there were no significant differences in the total cross-sectional area, marrow area or cortical cross-sectional area of the femoral shaft between any of the groups.

As for the ultimate load and stiffness of the femoral neck, alendronate at all dosage levels increased the ultimate load of the femoral neck compared with the value for the OVX group. The increase was significant at 0.04 and 1.0 mg/kg of alendronate, but no dose dependence was observed. Pamidronate and etidronate also tended to increase the ultimate load of the femoral neck, but these increases were not significant. Three bisphosphonates at relatively high doses also tended to increase the stiffness of the femoral neck, compared with the value for the OVX group. Alendronate and other bisphosphonates did not influence the geometric parameters of the femoral neck (length, area and angle of the femoral neck).

The correlation of femoral neck BMD (R5) with the ultimate load or stiffness of the femoral neck is shown in figure 3. The ultimate load and stiffness of the femoral neck were positively correlated with BMD of proximal end of the femur containing the femoral neck and trochanter (r = 0.263, P <
mg/kg but tended to increase it at a dose of 125.0 mg/kg. This finding suggests that etidronate impairs mineralization when given at 125 mg/kg. Alendronate decreased MAR to the level of the Sham group at doses of 0.2 mg/kg and above, whereas etidronate decreased MAR to a lower level than that of the Sham group at 125.0 mg/kg. There were no significant differences in LBG between the OVX and Sham groups. Alendronate did not affect LBG at all dose levels. Although etidronate statistically increased LBG at a dose of 25.0 mg/kg, there was no change from the OVX value for a 125.0 mg/kg dose.

Discussion

In this study, prominent decreases in BMD were observed at R1 (femoral distal epiphysis) and R5 (femoral neck and greater trochanter), but not at R3 (femoral diaphysis), after OVX. In the femoral epiphysis and neck, the effect of alendronate on BMD was observed even at a daily p.o. dose of 1.0 mg/kg. Although pamidronate prevented the bone mineral decrease, the effect was observed at 5.0 mg/kg only in the distal epiphysis. Etidronate did not exhibit any significant effects, even at 125.0 mg/kg. These findings suggest that the preventive action of alendronate toward bone mineral decrease was 5 times more potent than that of pamidronate and at least 125 times more potent than that of etidronate. These relative efficacy ratios agree with the experimental results of the antiresorptive activity of these drugs in a hypercalcemic model (Azuma et al., 1995) and toward the tibial metaphysis in growing rats (Schenk et al., 1986; Shinoda et al., 1983). Alendronate did not significantly increase BMD at R3 or the strength of femoral diaphysis. Bisphosphonates bind preferentially to bones that have high turnover rates, and their distribution in bones is not homogeneous (Lin et al., 1991; Azuma et al., 1995). This heterogeneous distribution could be the reason why alendronate is more effective in the epiphysis than in the diaphysis.

Bisphosphonates accumulate in the bone, so the biological half-lives of bisphosphonates are very long (Lin et al., 1991). In this experiment, the Ca/P ratio was not altered by alendronate treatment, even at a dose of 5.0 mg/kg. This result suggests that the composition of the mineral materials treated with alendronate for 2 months is similar to that in control rats.

The results on dry bone weight per bone volume and ash weight per bone volume were consistent with those on BMD found by DEXA. The preventive effects of alendronate on ash weight per bone volume were observed at doses lower than that found effective on BMD as measured by DEXA. These results suggest that the effects on ash weight per bone volume reflect the sum total of the small amounts of the change in each site of bone.

Alendronate given at 0.04 mg/kg significantly prevented BV/TV decrease at 1.0 mg/kg and above and reduced the ES/BS increase caused by OVX in a dose-dependent manner. These results suggest that the preventive effects of alendronate on the decrease in BMD were based on its inhibitory effect on bone resorption. It was reported that the initial rapid phase of trabecular bone loss of OVX rats is coincident with the maximal increase in bone turnover (Yamaura et al., 1996). An increase in bone turnover would result in bone loss so that an imbalance would exist between bone resorption

**Fig. 3.** BMD in R5 (which contains the femoral head, femoral neck and femoral greater trochanter) of the femur, measured by use of DEXA, was correlated with ultimate load of femoral neck (panel a) and with the stiffness of the femoral neck (panel b). The correlation between BMD and these two biomechanical parameters was estimated from rats of all groups except the sham group.

.05 for the ultimate load; \( r = 0.368, P < .01 \) for the stiffness). These correlations suggest that the BMD of the femoral neck reflects bone strength.

**Histomorphometric analysis.** The results of histological analyses on the proximal metaphysis of the right tibia for the alendronate and etidronate groups are shown in figures 4 and 5. The trabecular BV/TV (%) in the OVX group was significantly lower than that in the Sham group, which indicates that OVX causes a decrease in bone volume. The ES/BS (%), OV/BV (%), and MAR (millimeters/day) in the OVX group increased significantly relative to the Sham group. These results suggest that the effects on ash weight per bone volume were consistent with those on BMD.
and bone formation, with emphasis on the former process (Wronska et al., 1991). In this study, OV/BV and MAR, which reflect bone turnover, also increased after OVX, and alendronate inhibited these increases when given at 0.2 mg/kg and above. These decreases in OV/BV and MAR may depend on a decrease in bone turnover caused by the inhibition of bone resorption. However, the magnitude of both bone resorption and the suppression of its formation in alendronate-treated groups, even at 5.0 mg/kg, resulted in a level of bone turnover similar to that of the Sham group. Alendronate also did not have a significant effect on longitudinal bone growth. These results suggest that alendronate does not interfere with normal bone growth in growing rats. Etidronate at 25.0 mg/kg prevented the increases in the OV/BV, ES/BS and MAR to the same extent as alendronate, but it tended to increase OV/BV at a dose of 125.0 mg/kg. Furthermore, etidronate at 125 mg/kg significantly decreased MAR to a level lower than that of the Sham group. This finding suggests that etidronate impaired mineralization at higher doses. Because etidronate also prevented the decrease in BV/TV at 125.0 mg/kg, the
The dose level at which mineralization impairment appears is close to the dose level at which its antiresorptive activity appears. Lepola et al. (1996) reported that etidronate at 25 mg/kg/week (s.c. administration) impaired bone mineralization and decreased the bone formation rate below the control level in OVX rats, as was observed in the present study. Alendronate did not exhibit impairment of mineralization at the dose levels used in this study. This finding confirms that alendronate inhibits bone resorption without causing impairment of mineralization in growing rats, as previously reported (Schenk et al., 1986).

The effects of bisphosphonates can be considered at three levels: the tissue, the cellular and the molecular levels. However, the detailed mode of action of bisphosphonates has not been elucidated (Rodan and Fleisch, 1993). The difference in the effect on bone mineralization between these bisphosphonates may be attributed to the differences in their distribution profile in bone in vivo. After being distributed in bone tissue, and particularly on the surfaces of bone undergoing resorption, alendronate is thought to be released from the bone surface because of the acidic conditions formed by bone-resorbing osteoclasts, which enables it to act on osteoclasts (Rodan and Fleisch, 1996; Sato et al., 1991). Recently, Masarachia et al. (1996) reported that alendronate, at pharmacologically active doses, showed higher uptake on resorption vs. formation surfaces than etidronate. For $^3$H- etidronate, both osteoblast and osteoclast surfaces were labeled to a similar extent, whereas $^3$H-alendronate labeled 4.8% of osteoblasts and 37% of osteoclasts. Because the osteoblast surface of adult skeletons is larger than the osteoclast surface, this generates a large pool for the drug, and more etidronate would have to be given to achieve the necessary level of uptake by the osteoclasts. This pharmacodynamic factor could account for some, but not all, of the lower in vivo potency of etidronate vs. alendronate. The larger presence of $^3$H-etidronate on osteoblast surfaces might explain its greater propensity for inhibiting mineralization. Recently, Katsumata et al. (1995) reported that intermittent cyclical etidronate administration prevented bone loss in the vertebral body of OVX rats without impairing bone mineralization. This requirement for intermittent dosing to minimize the possibility of osteomalacia (Khari et al., 1974) limits etidronate’s general acceptance in the treatment of osteoporosis.

In this experiment, mechanical properties of femoral shaft and femoral neck were investigated. Alendronate did not significantly alter the breaking load or cross-sectional shape of the femoral shaft. Similar results were obtained for the femoral neck strength and femoral neck geometry. Although alendronate prevented the loss in BMD of femoral neck, any increase in femoral neck strength was not observed clearly. However, the ultimate load and stiffness of the femoral neck were positively correlated with BMD of the neck. Figure 3 shows the data of alendronate-treated groups located at the

![Graph](image_url)
upper-right position in the figure in comparison with that of the OVX group. This result indicates that the preventive effects of alendronate on femoral neck BMD reflect its preventive effects on femoral neck strength. These results show that continuous treatment with alendronate for 2 months did not decrease the biomechanical properties of rat femur. Alendronate did not affect the mechanical properties of the femur and vertebrae in adult dogs that were not subject to increased bone turnover (Chennekatu et al., 1996). On the other hand, Guy et al. (1993) gave alendronate p.o. to normal rats continuously for 2 years and found strikingly increased values of vertebral compression and femur bending. The treatment period of alendronate in our study might not be enough to increase the bone strength significantly. Similar results were obtained for pamidronate treatment and etidronate treatment in this study.

It has been reported that the geometry of the femoral neck affects the incidence of hip fracture (Nakamura et al., 1994). Sogaad et al. (1994) reported that there was no change in the neck-shaft angle with exercise or age, but there was a training-induced increase in femoral length and acceleration of skeletal maturation. We measured the architectural parameters to determine whether long-term daily administration of bisphosphonates would alter the architecture or geometry of the femoral neck in growing rats. Alendronate did not alter femoral neck length, neck-shaft angle or neck peripheral length. Furthermore, alendronate did not affect LBG in the histomorphometric observations. These results suggest that continuous treatment with alendronate did not interfere with bone growth and bone geometric properties. In this study, neither pamidronate nor etidronate changed these geometric parameters.

In conclusion, p.o. treatment with alendronate prevented a decrease in bone mineral and maintained the mechanical properties of bone after OVX without impairing bone mineralization in growing rats.

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References


