Prevention of Corticosteroid-Induced Bone Loss with Alendronate (44218)

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Abstract. **The putitive bone-sparing effect of alendronate was tested in two animal models of osteopenia: estrogen-deficient female rats and glucocorticoid-treated male rats. In the first study, 18 female Sprague-Dawley rats, 4 months of age, were ovariectomized (OVX), and an additional 6 rats were sham-operated. The OVX rats were treated with either vehicle, 17β-estradiol (E_2) (100 μg/rat/week, sc), or alendronate (1 mg/kg/day, on alternate days, orally). In the second study, 24 8-month-old male Wistar rats were treated with either vehicle, methyl prednisolone (7 mg/kg once a week, sc), prednisolone plus testosterone (16 mg/kg once every 3 weeks, im), or prednisolone plus alendronate (20 μg/kg twice a week, sc). Prior to treatment and at the end of the 6-week treatment period, bone mineral density (BMD) of the lumbar spine was measured by dual energy x-ray absorptiometry, and mean femur weights were calculated. The OVX rats had subnormal BMD (−3.91 ± 1.0% *vs* control +5.19 ± 3.92%, $P < 0.05$) and femur weights (720 ± 6 mg *vs* %; 746 ± 11 mg, $P < 0.05$). OVX-induced bone loss was completely abolished by the administration of E_2 (7.01 ± 2.32%, $P < 0.005$; 748 ± 6 mg, $P < 0.01$), or alendronate (24.2 ± 2.73%, $P < 0.0001$; 779 ± 11 mg, $P < 0.001$). In the second study in older male rats, glucocorticoids significantly decreased BMD (−9.70 ± 3.44% *vs* −1.10 ± 1.75%, $P < 0.05$), and femur weight (1070 ± 14 mg *vs* 1180 ± 24 mg, $P < 0.01$). Concomitant administration of testosterone (BMD 4.23 ± 1.84%, $P < 0.005$; femur weight 1260 ± 56 mg, $P < 0.02$), or alendronate (BMD 8.18 ± 1.36%, $P < 0.001$; femur weight 1360 ± 50 mg) with prednisolone, abolished the corticosteroid-induced bone loss. Bone histomorphometry showed a 34% loss of trabecular bone volume in glucocorticoid-treated rats ($P < 0.05$), which was prevented with both testosterone and alendronate therapies. However, at the doses used in both models, alendronate was more efficacious than either E_2 or testosterone in increasing BMD and femur weight. In summary, this study demonstrated that alendronate therapy is highly effective in counteracting the osteopenia of OVX and glucocorticoid therapy.**

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Osteoporosis is a major deleterious outcome of a number of endocrine disorders, such as estrogen deficiency (postmenopausal) and glucocorticoid therapy, as well as secondary to immunosuppression (i.e., rheumatological disorders, and post-transplantation, etc.) (1–7). Surgical or age-related menopause in women [in parallel with ovariectomized (OVX) primates and rats] leads to both cortical and trabecular bone loss (3, 4). The local mechanisms responsible for the development of osteopenia are poorly understood. Bone loss associated with diminished estrogen levels has been attributed to increased osteoclastic activity (3, 8, 9). Estrogen therapy presumably acts by inhibiting interleukins (IL) such as IL-1 and IL-6 (10, 11). Corticosteroids are well known to cause bone loss (i.e., osteopenia) (1, 5, 12, 13), and this is a major problem in clinical practice (2, 3, 7). The pathophysiology anti-anabolic effects of glucocorticoid-induced osteopenia is thought to be due to a direct decrease in bone formation (1–3, 12). Anti-anabolic effects of corticosteroids is thought to be due to disruption of cell-matrix integrity, osteoblast organization, and a possible decrease in the production and

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responsivity to cytokines and growth factors (14, 15). Periosteal osteoblast cultures have a sharply diminished capacity to express β-1 integrin mRNA following a 96-hr exposure to 10^{-7} *M* corticosterone (16, 17), and these cells show a deficient number of canalicular processes. Bone resorption also increases, and the changes are parathyroid hormone–dependent. New pharmaceutical tools appear to terminate excessive bone deposition. Clinical efforts are directed towards improving the quantity and the quality of the bone mass.

Alendronate is a potent bisphosphanate. It inhibits osteoblast-mediated osteoclast formation and osteoclastic activity (18, 20). These data are from studies in postmenopausal women and estrogen-deficient (i.e., oophorectomized) rats. The adverse effects of glucocorticoids on bone are well established (1–5, 21), but the beneficial effects of alendronate on glucocorticoid-induced osteoporosis (and its mechanisms of action) are yet to be determined. The current study was designed to investigate this issue. First, we confirmed, the actions of alendronate in an ovariectomized (OVX) rat model (a model for high bone turnover), and the glucocorticoid-induced bone loss (low bone turnover) in matured male rats (8, 21, 22). Herein, we report that alendronate treatment inhibits the decrease in bone mineral density (BMD) and femur weight associated with corticosteroid treatment in males, and increases BMD over and above the control values.

Materials and Methods

Ovariectomy. Twenty-four 14-week-old Sprague-Dawley female rats (Charles River, Wilmington, MA) weighing approximately 220 g each were used in this study. All rats were allowed free access to water and a pelleted commercial diet (Purina Mills Seeds Inc., Richmond, IN) containing 0.97% calcium, 0.85% phosphorus, and 1.05 IU/g of vitamin D_3. Six rats were sham-operated, and the remainder were bilaterally ovariectomized. OVX rats were randomly allocated into three groups of 6 rats. One group (positive controls) was injected immediately after the surgery and thereafter at weekly intervals with 17β-estradiol (E_2) (subcutaneous administration of estradiol cypionate 100 μg in 100μl of sesame oil) once a week (23), (Schein Pharmaceuticals, NJ). A second group (placebo controls) received only the sesame oil carrier (100 μl once a week). Group 3 received oral alendronate [1 mg/kg/every other day (corresponding to 0.5 mg/kg/day) administered by gavage feeding]. Food intake was withheld for 3 hr before and 1 hr after oral dosing of alendronate, but the rats were allowed free access to water.

Glucocorticoids. Twenty-four 32-week-old male Wistar rats (Charles River, Wilmington, MA), weighing approximately 340 g each, were used in this study. All rats were allowed free access to water and a pelleted commercial diet as in Experiment 1. The doses of medications used in this study were selected from previous work from others and ourselves (12, 18, 24–28). Rats in groups of six were allocated to receive a sc (subcutaneous) injection of either vehicle (placebo), methyl prednisolone (7 mg/kg/once a week, sc), methyl prednisolone plus testosterone (16 mg/kg once in 3 weeks, im); or prednisolone plus alendronate (sc of injection 20 μ/kg, twice a week). Testosterone was included in this protocol using male rats because it has been shown previously that corticosteroid therapy reduces circulating testosterone levels (29–31) and decreased testosterone levels are associated with lower BMD values (3, 4).

The doses of alendronate used in this study were based on the efficacy of this drug in humans and rats (6, 18, 19, 24, 25). In the second study, alendronate was administered subcutaneously to minimize the possible variabilities associated with oral administration. Both experimental protocols were approved by the Animal Care and Use Committee at The University of Texas Medical Branch at Galveston, and the animals were maintained in accordance with NIH Guidelines for the Care and Use of Laboratory Animals. Serum osteocalcium was measured using commercial ELISA (Diagnostic Systems Laboratory, Webster TX). The detection limit of the assay is 0.6 ng/ml.

Bone Mineral Density Assessment. Bone mineral density (BMD) was determined in the lumbar spine (L2-L4), as previously described using a QDR-1000W (Hologic Inc., Waltham, MA) dual energy x-ray absorptiometry (DXA) bone scanner at the beginning and 6 weeks after therapy (23, 32). The machine was adapted for an ultra high-resolution mode with line spacing of 0.0254 cm, point resolution of 0.0127 cm [which increased the number of lines scanned (4×) and slowed the speed of the scanning arm to produce an over sampling that led to an increase in point resolution (7.5×)], and x-ray collimator diameter of 0.9 mm (23, 32). Animals were anesthetized beforehand by intraperitoneal injection of ketamine HCl (40 μg/kg body weight) and xylazine (10 μg/kg). To minimize variabilities, the BMD (g/cm^2; derived by dividing the measured bone mineral content by the area of the bone scanned) was measured in 0.138 cm^2 in the center of each L2 to L4 vertebrae, and the mean values were calculated. The results are expressed as the percentage change of BMD (mean ± SEM).

Bone Histomorphometry and Femur Weights. An automated computerized histomorphometric package (Optimas Corp., Bothwell, WA) was used to measure: 1) the trabecular bone volume (%) in the distal femoral metaphysis and proximal tibial metaphysis, and 2) cross-sectional mid-shaft femoral and tibial cortical bone volumes (mm^2). The slides were coded, and all measurements were performed at a magnification of 64× by the same person blinded to the identity of the groups. The data were expressed in terms of the mid-shaft cortical bone area of the femur (mm^2) and percent trabecular bone volume (%TBV) of the proximal tibia (6, 8, 20, 33). At the end of the experimental period, animals were sacrificed, and mean weights of the femurs (mg) were obtained.

Statistical Analysis. The results were expressed as the means ± SEM. For between group comparisons, the

changes of BMD were calculated from the baseline measurements and expressed as percentages as a measure of efficacy. For the assessment of differences between treatment groups, ANOVA was used with multiple comparisons with Wilcoxon rank sum test. The baseline lumbar BMD data between groups were also analyzed by ANOVA. In the histomorphometric data, the differences between the means were analyzed by a non-parametric two tailed *t* test and Wilcoxon Rank order statistics. Differences between means at the 5% confidence level ($P < 0.05$) were considered to be statistically significant.

Results

Ovariectomy Study. Among the four groups of rats studied, there were no significant differences of BMD observed at the baseline. Figure 1 illustrates the percentage changes of BMD in the lumbar spine 6 weeks after each therapy as determined by DXA. The changes of BMD in these growing OVX rats were substantially lower (−3.91% ± 1.00) in comparison to sham-operated rats (5.19% ± 3.92; $P < 0.05$). Both E_2 (7.01% ± 2.32, $P < 0.005$) and alendronate (24.2% ± 2.73, $P < 0.0001$) therapies completely abolished the OVX-induced bone loss. The alendronate group also had a significantly higher BMD in comparison to sham-operated rats ($P < 0.01$); treatment produced a 19.0% increase in BMD, which exceeded the control rats.

Figure 2 shows the weights of femurs in rats following various treatments for 6 weeks. Femoral weights in OVX rats were substantially lower (720 ± 6 mg) compared to sham-operated rats (746 ± 11 mg, $P < 0.05$). The OVX-induced decrease in femoral weight was reversed by both E_2 (748 ± 6 mg, $P < 0.01$) and alendronate (779 ± 11 mg, $P < 0.001$). In comparison to sham-operated rats, alendronate-treated rats had significantly higher femoral bone weights (4.4%; $P < 0.05$).

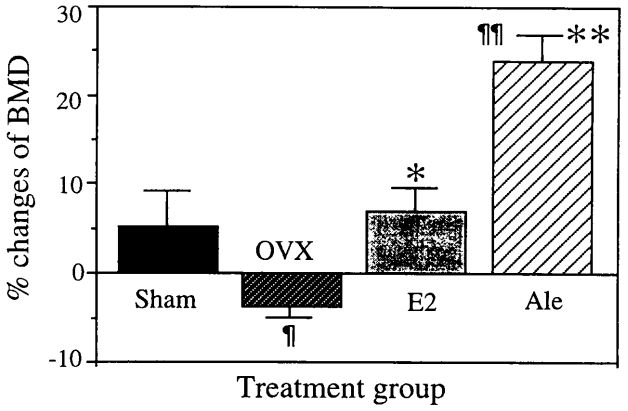

Figure 1. Percentage change in bone mineral density (BMD) over the 6-week treatment period in various treatment groups: Sham, sham-operated; OVX, ovariectomy (control); E_2, estrogen-treated; Ale, alendronate-treated. Values represent the mean ± SEM for six animals per group. Comparisons were made against reference rats (sham control): ¶$P < 0.05$, ¶¶$P < 0.01$. Comparisons were made against ovariectomized rats (OVX control): *$P < 0.005$, **$P < 0.0001$.

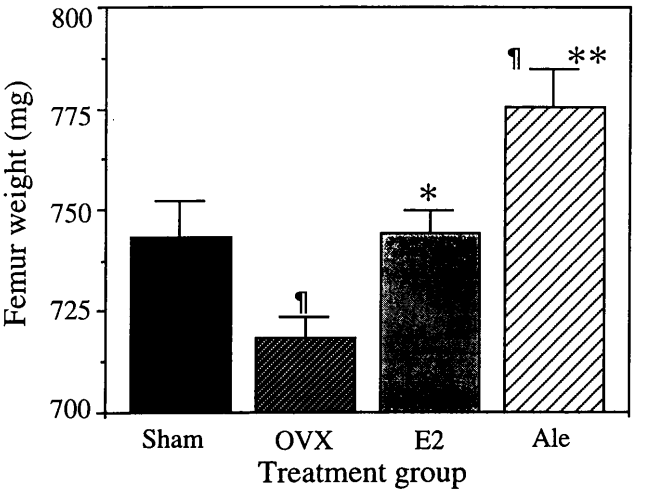

Figure 2. Changes in femur weights (mg) over a 6-week treatment period in various treatment groups: Sham, sham-operated; OVX, ovariectomy, E_2, estrogen-treated; Ale, alendronate-treated. Values represent the mean ± SEM of right and left femurs from six animals per group. Comparisons were made against reference rats (sham control): ¶$P < 0.05$. Comparisons were made against ovariectomized rats (OVX control): *$P < 0.01$, **$P < 0.001$.

Glucocorticoid Study. Among the four groups of male rats studied, baseline lumbar BMD values were similar. Figure 3 illustrates the percentage changes of BMD 6 weeks after each therapy as determined by DXA. In comparison to the vehicle-treated control rats (−1.10% ± 1.75), the glucocorticoid-treated rats showed a substantial decrease in BMD (−9.70% ± 3.34, $P < 0.05$). Both testosterone (4.23% ± 1.84, $P < 0.005$) and alendronate (8.18% ± 1.36, $P < 0.0001$) therapies prevented bone loss.

Figure 4 shows the weights of femurs in rats following various treatments for 6 weeks. Femoral weights in corticosteroid-treated rats were significantly lower (1070 mg ±

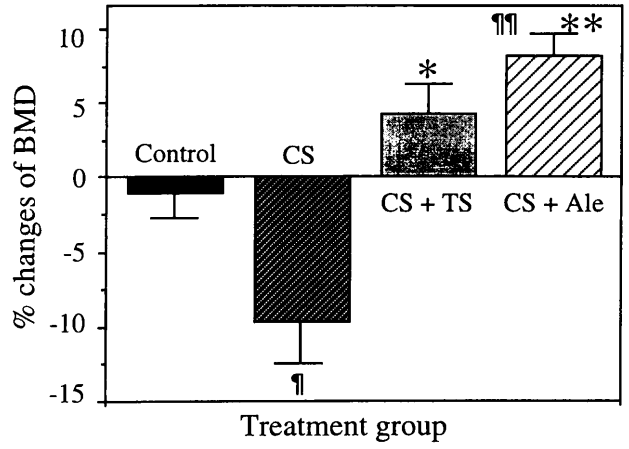

Figure 3. Percentage change in bone mineral density (BMD) over a 6-week treatment period in various treatment groups: control (placebo treatment); CS, corticosteroids; CS + TS, the combination of corticosteroids plus testosterone; and CS + Ale, the combination of corticosteroids plus alendronate. Values represent the mean ± SEM for six animals per group. Comparisons were made against the control (reference rats): ¶$P < 0.05$; ¶¶$P < 0.01$. Comparisons were made against corticosteroid-treated rats (CS): *$P < 0.005$, **$P < 0.0001$.

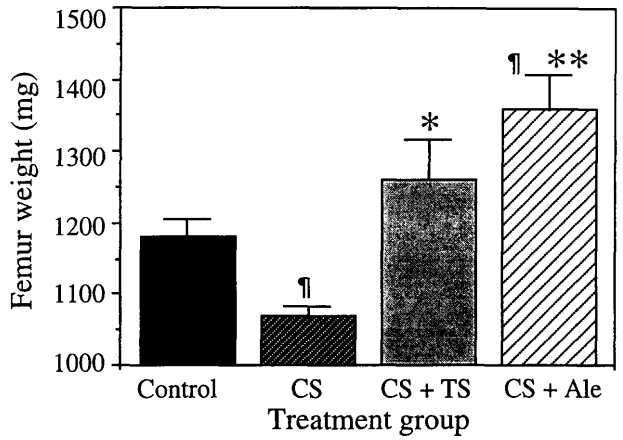

Figure 4. Changes in femur weights (mg) over the 6-week treatment period in various treatment groups: control (placebo treatment); CS, corticosteroids; CS + TS, the combination of corticosteroids plus testosterone; and CS + Ale, the combination of corticosteroids plus alendronate. Values represent the mean ± SEM for six animals per group. Statistical comparisons were made against the control (reference rats): ¶$P < 0.01$. Comparisons were made against corticosteroid-treated rats (CS): *$P < 0.02$, **$P < 0.002$.

14) compared to control rats (1180 ± 24 mg, $P < 0.01$). This corticosteroid-induced decrease in femoral weight was prevented by treatment with testosterone (1260 ± 56 mg, $P < 0.02$) and alendronate (1360 ± 50 mg, $P < 0.002$). Rats treated with alendronate had a significant increase of their lumbar spine BMD ($P < 0.0001$) and femur weights ($P < 0.002$) in comparison not only to glucocorticoid-treated rats, but also to the vehicle-treated control rats (9.3% increase of BMD, $P < 0.005$; and 15.2% increase of femur weights $P < 0.02$).

Figure 5 shows serum osteocalcin levels in four groups of rats. In comparison to the values obtained in control rats, the levels of serum osteocalcin were decreased in rats treated with corticosteroids. While the changes were not statistically significant ($P = 0.1$), one would expect lower osteocalcin in rats with evidence of low bone formation. Rats that received testosterone (in addition to prednisolone) had similar levels of serum osteocalcin to those found in control rats. Alendronate therapy did not significantly affect serum osteocalcin levels.

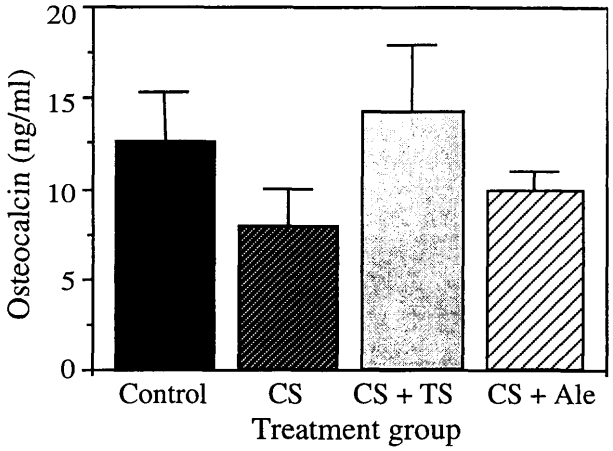

Figure 5. The levels of serum osteocalcin (ng/ml) in various treatment groups: control; CS, corticosteroids; CS + TS, the combination of corticosteroids plus testosterone; CS + Ale, the combination of corticosteroids plus alendronate. There were no statistical differences (ANOVA) between the groups.

Figure 6 shows the bone histomorphometric data from the mid-shaft cortical area (mm^2) of the femur and percentage TBV of the proximal tibia. The cortical bone area of rats treated with corticosteroids was 18% lower than the control group, but statistically insignificant [$P = 0.24$, Fig. 6A]. However, the group treated with corticosteroids had a 34% lower TBV ($P < 0.05$), whereas the group treated with testosterone and alendronate did not lose trabecular bone.

Discussion

The osteopenia induced by OVX and prednisone therapy in rats has been widely used as a model of human osteoporosis (8, 9, 23, 34). Our data in the OVX rats confirmed that treatment with either estrogen or alendronate prevents OVX-induced bone loss in adult female rats. This study also confirms the effective dosage of alendronate on BMD in OVX rats (26). The percentage increase in BMD

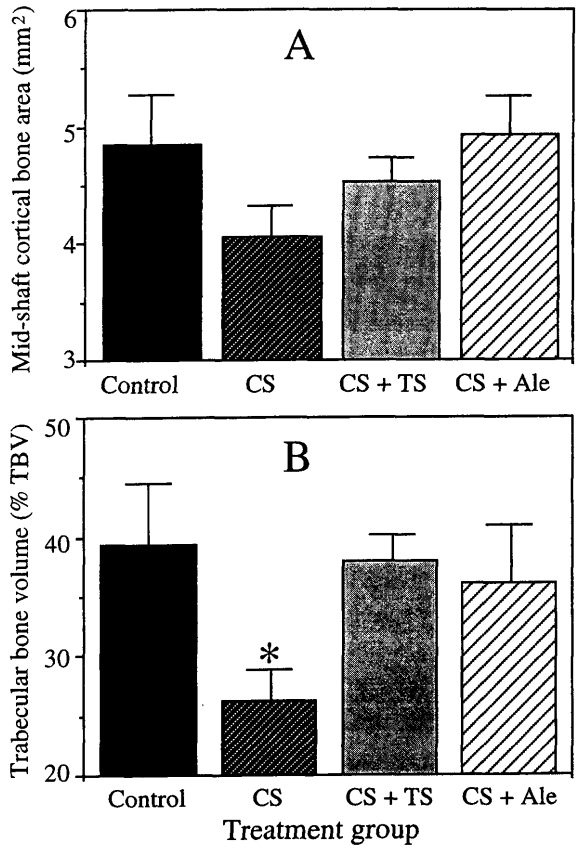

Figure 6. Changes of mid-shaft cortical bone area (mm^2) of the femur (A) and trabecular bone volume (B) (%TBV) in the proximal tibial metaphysis in four treatment groups: control (placebo); CS, prednisolone (corticosteroids); CS + TS, the combination of corticosteroids plus testosterone; CS + Ale, the combination of corticosteroids plus alendronate. Values are means ± SEM for three to five animals per group. Statistical comparisons were made against the control group (ANOVA): *$P < 0.05$.

observed with alendronate was higher than that obtained by E_2 replacement therapy.

The study in prednisone-treated rats shows that alendronate therapy could benefit individuals on high dose prednisolone regimens. This study demonstrated that corticosteroid therapy in rats significantly decreased the BMD. Testosterone or alendronate prevented this osteopenia. After alendronate therapy, the increase of BMD and femur weights observed were significantly higher than that seen with testosterone. There was a substantial decrease in both BMD and femoral weights in OVX and corticosteroid-treated rats compared with rats treated with either E_2 and testosterone, or with alendronate. This study shows that alendronate has a therapeutic value in preventing the bone loss due to estrogen deficiency as well as corticosteroid therapy.

Alendronate is a potent bisphosphonate which inhibits osteoclastic bone resorption (18–20, 26). This drug decreases bone resorption in conditions associated with increased bone turnover (18, 24, 26), or decreased bone turnover (e.g., skeletal unloading) (27). This occurs *via* the inhibition of osteoblast-mediated osteoclast formation (20, 35). Alendronate decreases the bone loss associated with oophorectomy and that induced by prostaglandin (20, 35), thyroxine (24), and parathyroid hormone (19). These studies have shown that this drug is capable of improving the histomorphometric and biochemical properties of bone in OVX rats (18, 20).

A variety of drugs have been evaluated as therapeutic agents for the prevention and treatment of glucocorticoid-induced osteoporosis. These include vitamin D (5), estrogen and progesterone (36), growth hormone (22), and a triple therapy (sodium fluoride, calcium, and vitamin D) (12), but results are variable. Our data demonstrate that treatment with alendronate protects against corticosteroid-induced bone loss in rats (Figs. 3 and 4). The data obtained with bone histomorphometry are compatible with BMD data and confirmed the beneficial effects of alendronate on bone. Bone histomorphometry showed a normalization of mid-shaft cortical bone area and TBV% in both testosterone and alendronate-treated rats, but BMD and femur weights showed an increase above and beyond the control. This is probably due to hyper-mineralization secondary to these two therapies. While the decrease in serum osteocalcin levels were not statistically significant, perhaps due to small sample number, the trend was to be expected in view of the deleterious effects of glucocorticoids on the rate of bone formation.

If the rat data hold in humans, these studies suggest that therapy with both testosterone and alendronate may prevent corticosteroid-induced bone loss. Patients who are receiving chronic glucocorticoid therapy (as with asthma, connective tissue disorders, rheumatic diseases, post-transplantation, etc.), would be at special risk. At the doses used in these two studies, alendronate not only significantly increased both BMD and femur weights and preserved the bone quality (as assessed by maintenance of the static histomorphometric measures) in both OVX and corticosteroid-treated rats, but also was more efficacious than E_2 and testosterone in increasing BMD and femur weight.

The current study demonstrates that administration of alendronate or testosterone may have a therapeutic value in preventing bone loss in patients who are being treated with corticosteroids. In summary, we have demonstrated that alendronate treatment can attenuate the OVX-induced and glucocorticoid-induced bone loss in rats. We confirmed this beneficial effect of alendronate on bone by using various parameters (i.e., BMD, femoral weights, and histomorphometry) and illustrated the potent effects of alendronate in the prevention of corticosteroid-induced bone loss.

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