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EFFECTS OF ONE TO THREE YEARS TREATMENT WITH ALENDRONATE ON MECHANICAL PROPERTIES OF THE FEMORAL SHAFT IN A CANINE MODEL: IMPLICATIONS FOR SUBTROCHANTERIC FEMORAL FRACTURE RISK

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Abstract

Bisphosphonate (BP) treatment used to prevent bone loss in postmenopausal osteoporosis has recently been implicated in an apparent increase in subtrochanteric femoral fractures. Previous work has shown that BPs can reduce the energy to fracture of cancellous bone, but there are limited data on material-level mechanical properties of compact bone from the long bones. This study examined intrinsic mechanical properties of the femoral diaphysis of a canine model treated for one or three years with alendronate at two different doses. Seventy-two dogs were treated orally with 0.2 mg/kg/day alendronate or 1.0 mg/kg/day alendronate; a control group was administered saline. Prismatic beam specimens were tested in 4 point bending under displacement control and the intrinsic mechanical properties were calculated. There were no significant differences among groups in any mechanical property at either 1 or 3 years of treatment. We conclude that the material properties of the femoral diaphysis are not degraded following 1-3 years treatment with alendronate, even at high doses. Although longer periods of treatment have not been studied using clinical doses of alendronate, such studies need to be carried out in order to confirm a lack of effect of alendronate on mechanical properties of cortical bone in the subtrochanteric region of the femur.

Keywords

Subtrochanteric fractures; Femur; Bisphosphonates; Biomechanics

We have extensively documented changes to mechanical properties of canine bone following a 1-3 year treatment with clinical doses and higher doses of the oral bisphosphonates alendronate (ALN) or risedronate (RIS).[1-5] These reports show a tendency for canine bone treated with bisphosphonates to fail at lower energy than untreated bone, when adjusted for changes in bone mineral density.[6-7] Toughness, the material energy to fracture, has consistently been shown to decrease by 15-20% in vertebrae following one year treatment with clinical doses of ALN,[3] and to continue to decline over a 3 yr treatment period by nearly 30% in both vertebrae and ribs compared to animals not treated with ALN.[4,5] The pre-yield material properties of the bone - strength (yield stress) and elastic modulus - do not change significantly

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over the treatment period, but smaller post-yield displacement prior to fracture underlies a significant reduction in the post-yield toughness.[5] A reduction in energy to failure following the yield point is consistent with a brittle fracture.

The reasons for this embrittlement are not entirely clear. It has been suggested that this is associated with increased microdamage accumulation,[1-3] higher overall tissue mineralization as the result of the older mean tissue age of bone,[8-12] and/or changes to the collagen and its cross-links that involve the accumulation of advanced glycation end-products known to make bone brittle.[13-18]

Recently, several articles have appeared in the scientific literature reporting what appears to be an increased incidence of subtrochanteric femoral fractures in the osteoporotic population. Most of those who fractured had been treated for more than four years with alendronate.[19-25] These cortical bone fractures occurred with minimal trauma, a typical feature of low energy fractures. Morphologically, the fractures showed characteristics similar to those of a brittle stress fracture. The fracture line is often transverse, rather than spiraling, and in some cases there is evidence of periosteal woven bone typical of that seen in a healing stress fracture. Interestingly, Lenart et al.[23] found that the majority of these fractures are associated with cortical thickening of the femoral shaft. One difficulty in associating subtrochanteric femoral fractures with bisphosphonate use is that there are a significant number of co-morbidities (eg diabetes, glucocorticoid therapies) in those who fracture.[22]

The effects of bisphosphonate treatment on low turnover cortical bone of the extremities have not been studied in any detail. The purpose of the current work was to measure the intrinsic mechanical properties of femoral cortical bone in dogs treated for 1 or 3 years with alendronate at a dose used to treat postmenopausal osteoporosis, or at a dose 5x greater than this. The higher dose can provide a worst case scenario that could mimic treatment at lower doses but for longer periods of time.

METHODS

Animals and Experimental Design

The details of this set of experiments have been described several times.[3,4] Data in this paper are reported from 72 skeletally mature female dogs (n = 12/treatment group; Marshall Farms, USA, North Rose, NY; and LBL Laboratories, Reelsville, IN) treated with alendronate (ALN, Merck and Co., Inc.) mixed with saline and administered orally by syringe at a dose of either 0.2 mg/kg/day (the dose on a mg/kg basis used to treat osteoporosis in women) or 1.0 mg/kg/day (five-fold the clinical dose for osteoporosis). Dogs were treated for either one or three years. Separate groups treated for either 1 or 3 years with saline (1 ml/kg/day) were used as controls. Dosing was performed each morning after an overnight fast at least 2 hours prior to feeding. Prior to sacrifice, animals were injected with calcein (5 mg/kg as a 3% solution, IV) using a 2-12-2-5 labeling schedule to allow measurement of dynamic histomorphometry. Three animals in each of the groups treated for 1 year were injected on a 2-5-2-5 day labeling schedule due to an error. After 1 or 3 years, animals were euthanized by i.v. administration of sodium pentobarbital (0.22 mg/kg Beuthanasia-D Special) and femurs were dissected free, wrapped in saline-soaked gauze and frozen (-20° C.). All procedures were approved by the Indiana University School of Medicine Animal Care and Use Committee prior to the study.

Specimen Preparation

Under constant irrigation, a 30 mm length of bone from the midshaft of the femur was removed using a band saw, and two prismatic beams were cut along the longitudinal axis of the anterior and posterior cortices using a diamond embedded wire saw (Histosaw, Delaware Diamond

Knives, Wilmington, DE). The specimens were ground and polished to achieve the final dimensions of 25 mm × 1.8 mm × 1.5 mm thick (1 year specimens) or 26 mm × 1.8 mm × 1.4 mm thick (3 year specimens).

Densitometry

Areal bone mineral density (aBMD, g/cm^2), bone mineral content (BMC, g), and area (cm^2) of the machined cortical bone beams were quantified using a PIXImus II densitometer (Lunar Corp). Prior to scanning, the bones were thawed to room temperature. Software provided coefficient of variations for ex vivo scans are 0.5% for BMD and 0.6% for BMC.

Measurements of cortical thickness were made from DXA images obtained on the whole femora prior to machining of the beams. Images were available for measure for only a subsample of dogs treated for 3 years (VEH, n = 7; ALN 0.2, n = 7; ALN 1.0, n = 5). Thicknesses of the medial and lateral cortices were made at a distance of 9 mm distal to the lesser trochanter, and also at a distance of 27 mm from the lesser trochanter. Two measurements were made at each of these four locations, and the two measurements at each location averaged to obtain a single value.

Mechanical testing

The beams were subjected to monotonic four-point bending under displacement control (3 mm/min) and data collected at 10 Hz (EuduraTEC, Bose Electoforce 3200, Eden Prairie, MN). Intrinsic (material) properties (ultimate stress, elastic modulus, toughness) were estimated using standard formulae,[4,26] which normalizes for small variations in specimen dimension. Yield was determined by the 0.2% offset method.

Histomorphometry

Following testing, cross-sections, 80-100 μm thick, were prepared from the tested beams using a diamond wire saw (Histosaw; Delaware Diamond Knives, Wilmington, DE). Five to ten unstained sections were mounted on glass slides and fluorochrome labels were assess using a semi-automatic image analysis system (Bioquant OSTEO 7.20.10; Bioquant Image Analysis, Nashville, TN) attached to a microscope equipped with an ultraviolet light source (Nikon Optiphot 2; Nikon, Tokyo, Japan). The distance between sets of double calcein labels was measured and divided by interlabel period (12 days) to determine mineral apposition rate (MAR). The total length of all labeled surfaces (L.Pm) was measured, and from these parameters, bone formation using bone volume as a referent (BFR/BV) was calculated as (L.Pm × MAR)/BV.

Statistics

Our intent was not to assess the effects of treatment duration in this study, therefore differences among groups were assessed using one-way Analysis of Variance (ANOVA, SAS Institute, Inc) within a given treatment time. When the overall F-value was < 0.05, multiple comparisons among groups were evaluated using a Fisher's protected least-significant difference (PLSD) test. For all tests, $p < 0.05$ was considered statistically significant. All data are presented as mean ± standard error.

RESULTS

There were no differences in mineral density between the animals treated with ALN and vehicle-treated controls within either the one or three year time points. (Table 1). There were no significant differences among treatment groups in any material property within a given time

period of treatment (1 or 3 years). Likewise, we were not able to identify any differences in either pre-yield or post-yield toughness among the groups (Table 1).

Bone formation rate was calculated in the cortical bone of the beam specimens. VEH-treated animals had a BFR/BV of 1.10 ± 0.62%/year. Due to the paucity of label in ALN-treated animals (i.e. many animals had no label in any of the assessed sections) calculations of BFR/BV were not made.

Cortical thickness was measured on the medial and lateral cortices of the femur at both proximal and midshaft regions. We found no evidence of any differences among groups in cortical thickness at any of the four sites (data not shown).

DISCUSSION

The results of this study provide evidence that clinical or high-dose treatment for three years with alendronate does not adversely affect the material properties of femoral cortical bone. This observation achieves a level of importance when viewed in light of the recent reports of subtrochanteric fractures in osteoporotic women. The suggestion has been made that these fractures are found at higher than usual rates in the subpopulation of women treated with bisphosphonates. Indeed, we have previously reported a reduction in energy to fracture of nearly 20% in vertebral bone of these same dogs, following 1 year treatment with ALN at doses used to treat postmenopausal osteoporosis,[3] and an even greater reduction in bone toughness when treatment periods last longer.[4] Similar or greater reductions in toughness in vertebral bone have been noted using other bisphosphonates (eg incadronate) at high doses (2.5x the dose for osteoporosis) for three years.[27] Therefore, it was not unreasonable to be concerned that similar changes in bone in the subtrochanteric region of the femur could be associated with the increased risk of fracture.

Although mechanical testing of bone can be associated with high variability, making it difficult to detect changes in properties without large sample sizes, four-point bending studies of prismatic beams from dog femurs are sufficiently sensitive to identify differences among treatment groups when they exist. Four-point bending tests of beams machined from femurs of dogs treated with raloxifene, a SERM, established that raloxifene treatment was associated with a greater than 75% increase in femoral energy to failure and toughness, accounted for by a significant increase in post-yield displacement.[28] The fact that no changes occurred in the properties of femoral shaft cortical bone following treatment with ALN, therefore, is strong evidence that the diaphysis of the femur is relatively unaffected mechanically by a prolonged period of ALN treatment even at higher than clinical doses.

In the absence of estrogen-deficiency, turnover of cortical bone is typically slow. Parfitt[29] has estimated that bone turnover rates in human cortical bone averages about 3%/yr, compared to 30%/yr for cancellous bone. These data are from human rib biopsies; to our knowledge, there are no comparable data on bone turnover in the human femur. We calculated that turnover of cortical bone in these beams from vehicle treated animals was 1.10 ± 0.62%/year. This is very similar to turnover rates in cortical bone from the femoral neck, which range between 0.75% and 1.5% per year.[30] Given these low values for turnover rate, even without alendronate treatment, it may be no great surprise that the properties of the bone tissue in this region were not changed significantly even by a prolonged period of treatment with a high dose of alendronate. Even with a statistically significant decrease in bone turnover rate following treatment with ALN, the rate is so low that few effects would likely be detected. Although ALN at these doses clearly has an effect on trabecular bone turnover in the femoral neck,[31] it does not seem to have a significant effect on bone in the femoral diaphysis, even at doses that are five times higher than those normally given to women with postmenopausal osteoporosis.

Data on the effects of bisphosphonates in cortical bone are equivocal. Changes were observed in toughness of rib cortical bone following one or three years treatment at high doses of alendronate,[1,5] but not at lower doses. Likewise, post-yield work to fracture was significantly reduced by 28% in the tibia of these same dogs treated for one year at the high dose of ALN, but not those treated at the lower dose.[32]. Komatsubara et al.[33] found no changes in material properties in rib cortical bone following 3 years with doses of incadronate that were 2.5x or 5x the clinical dose. This suggests that, although cancellous bone material properties may be negatively impacted by bisphosphonate treatment, cortical bone may not be affected in this manner, at least at doses used for the clinical treatment of osteoporosis.

It has been reported that cortical thickness of the subtrochanteric region of the human femur is increased in those people presenting with subtrochanteric fractures following bisphosphonate treatment.[3] To investigate whether a similar change in cortical thickness occurred in this animal model, we measured cortical thickness in a subset of animals treated for 3 years. We were not able to find a significant effect of ALN on cortical thickness.

We cannot rule out that prolonged treatment with alendronate in people could have deleterious effects on the properties of the femoral diaphysis. However, the results from our studies using the canine model indicate that very high doses of alendronate given to an animal with an already low rate of cortical bone turnover for up to three years does not compromise the material properties of the femur. Yet we know that accumulation of microdamage and significant changes in collagen in the vertebrae occur within 1 year of treatment with both doses of either alendronate or risedronate.[2,3,17] Significantly increased glycation of the cortical bone of the tibia also occurs within one year of treatment at the higher drug doses.[32] Clearly, changes to the bone matrix can occur within the time frame of this study, even in cortical bone.

The results of our studies, therefore, suggest that the increased incidence of subtrochanteric fractures observed in women treated with bisphosphonates may be accelerated by co-morbidities or require a longer period of treatment than we were able to provide in this study. It is true that many of those who presented with subtrochanteric fractures had other conditions (eg diabetes) or were being treated with glucocorticoids.[20-22, 25]

There are several limitations to our study. First, the dogs used in this study were not estrogen-deficient and did not have low bone mass. However, the fact that they were not osteopenic should have no bearing on the calculation of intrinsic properties following ALN treatment. Second, we were only able to treat the dogs for up to 3 years, due to economic constraints. Those women who presented with subtrochanteric fractures and were taking alendronate had been taking the bisphosphonate for a mean of 4-5 years,[20,22,23] or 7 years in another study, [24] although several had taken it for 10 years or more.[25] Still, some presented with fractures following only 2-2.5 years of alendronate treatment at doses 5x lower than the high dose we gave in our study.[22] Although we know that these doses of bisphosphonates impart significant changes to the skeleton within the time period of this study, even in cortical bone, we acknowledge that the changes may take longer in the cortical bone of the femur. We have tried to address this in part by using very high doses for shorter periods of time than therapy lasts in humans being treated for osteoporosis. We do not know that this is an accurate reflection of what occurs with longer duration treatments, but do have data to suggest that higher doses will have significant effects on the bone earlier, providing a longer effective treatment time. Previously, we showed that the lower (0.2 mg/kg) ALN dose did not significantly suppress intracortical remodeling in rib cortical bone compared to vehicle-treated controls (VEH) after 1 year of treatment,[34] although it did have significant effects in rib by 3 years.[5] Although not reported in that paper, the higher dose (1.0 mg/kg) did significantly suppress remodeling at 1 year, as we also showed in an earlier paper.[1] Third, we do not know the rate of bone turnover in the proximal half of the femoral diaphysis of humans, nor how it compares with that in dogs.

If it is normally much faster than in dogs, and is suppressed to a greater extent with bisphosphonate treatment, this could underlie the observation of increased subtrochanteric fracture risk. However, if subtrochanteric fractures are a function of "oversuppression" with bisphosphonates, which has been the implication of previous work in humans, then using an intact dog which has low turnover in the femur even without bisphosphonate treatment, and suppressing turnover further using BPs, provides a reasonable test of whether significant suppression of turnover can lead to deleterious changes in bone's mechanical properties.

We conclude that there is no evidence that the material properties of the femoral diaphysis are altered following 1-3 years of treatment with daily oral alendronate, even at doses that are 5x higher than those used to treat postmenopausal osteoporosis.

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Table 1

Femoral Diaphysis Cortical Beam Properties

	1 year				3 year			
	VEH	**ALN 0.2**	**ALN 1.0**	**ANOVA**	**VEH**	**ALN 0.2**	**ALN 1.0**	**ANOVA**
aBMD, g/cm^2	0.121 ± 0.001	0.121 ± 0.001	0.121 ± 0.001	.8995	0.150 ± 0.002	0.148 ± 0.002	0.149 ± 0.003	.9096
BMC, g	0.084 ± 0.001	0.084 ± 0.001	0.084 ± 0.001	.9548	0.117 ± 0.002	0.116 ± 0.002	0.114 ± 0.002	.6756
Area, cm^2	0.698 ± 0.005	0.690 ± 0.006	0.693 ± 0.005	.6329	0.793 ± 0.011	0.793 ± 0.011	0.793 ± 0.008	.9977
Ultimate Stress, MPa	237 ± 9	251 ± 13	248 ± 12	.6823	329 ± 11	321 ± 17	317 ± 16	.8485
Elastic Modulus, GPa	33.4 ± 1.0	34.4 ± 1.5	33.7 ± 1.5	.8492	33.2 ± 1.4	29.9 ± 1.6	31.7 ± 1.5	.3061
Toughness, mJ/mm^3	1.56 ± 0.21	1.62 ± 0.17	1.85 ± 0.26	.6256	2.06 ± 0.23	2.55 ± 0.28	1.89 ± 0.36	.2801
Pre-yield toughness, mJ/mm^3	0.52 ± 0.02	0.56 ± 0.04	0.52 ± 0.02	.5774	0.46 ± 0.03	0.38 ± 0.03	0.43 ± 0.03	.2416
Post-yield toughness, mJ/mm^3	1.04 ± 0.22	1.06 ± 0.17	1.33 ± 0.27	.5925	1.60 ± 0.23	2.16 ± 0.27	1.46 ± 0.34	.1959