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Duration-dependent effects of clinically relevant oral alendronate doses on cortical bone toughness in beagle dogs

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Abstract

Bisphosphonates (BPs) have been shown to significantly reduce bone toughness in vertebrae within one year when given at clinical doses to dogs. Although BPs also reduce toughness in cortical bone when given at high doses, their effect on cortical bone material properties when given at clinical doses is less clear. In part, this may be due to the use of small sample sizes that were powered to demonstrate differences in bone mineral density rather than bone's material properties. Our lab has conducted several studies in which dogs were treated with alendronate at a clinically relevant dose. The goal of this study was to examine these published and unpublished data collectively to determine whether there is a significant time-dependent effect of alendronate on toughness of cortical bone. This analysis seemed particularly relevant given the recent occurrence of atypical femoral fractures in humans. Differences in the toughness of ribs taken from dogs derived from five separate experiments were measured. The dogs were orally administered saline (CON, 1 ml/kg/day) or alendronate (ALN) at a clinical dose (0.2 mg/kg/day). Treatment duration ranged from 3 months to 3 years. Groups were compared using ANOVA, and time trends analyzed with linear regression analysis. Linear regressions of the percent difference in toughness between CON and ALN at each time point revealed a significant reduction in toughness with longer exposure to ALN. The downward trend was primarily driven by a downward trend in post-yield toughness, whereas toughness in the pre-yield region was not changed relative to CON. These data suggest that a longer duration of treatment with clinical doses of ALN results in deterioration of cortical bone toughness in a time-dependent manner. As the duration of treatment is lengthened, the cortical bone exhibits increasingly brittle behavior. This may be important in assessing the role that long-term BP treatments play in the risk of atypical fractures of femoral cortical bone in humans.

Keywords

Bisphosphonates; Atypical femoral fractures; Toughness; Cortical bone

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Introduction

Alendronate (ALN), a bisphosphonate (BP), improves bone strength by reducing bone turnover (~70%) and increasing bone density, but evidence from animal studies [see [1] for review] and human case control studies [see [2,3] for review] suggests that this may come at the expense of impaired material properties. It is well established that 1–3 years treatment with oral ALN [4] or other BPs [5] is associated with a reduction in cancellous bone toughness, a material measure of energy to failure, even at clinical doses [6–8]. Similar reductions in tissue toughness can be inferred from recent data in ovariectomized nonhuman primates [9]. In that study, energy absorption in the lumbar vertebrae of ALN treated primates was similar to that in OVX animals, but BV/TV was higher [Mashiba, pers. comm.], implying reduced toughness.

The data regarding impaired mechanical properties in cortical bone is less convincing, but in light of recent evidence of atypical femoral fractures in cortical bone especially after prolonged treatment with BPs, such data are of extreme importance. The first study to show in an animal model that one year of treatment with ALN had negative effects on cortical bone toughness of the rib [4] used doses of ALN that were five times higher than those given clinically. A later study [10] confirmed that finding following three years of high dose ALN. Three-point bending of cortical beams from the tibia of dogs treated for one year with a high dose of ALN or risedronate (RIS) also resulted in significantly lower post-yield toughness [11]. High dose treatment with zoledronate (ZOL) reduced rib toughness after 9 months [12]. On the other hand, neither RIS [13] nor incadronate [14] were associated with a significant reduction in rib toughness following 1–3 years of treatment at 2.5–5x the clinical doses. The data seem clear that high dose ALN administration is associated with reductions in cortical bone toughness following 1–3 years of treatment, although other BPs may not be implicated.

At clinical doses, although ALN resulted in a 19% decrease in rib cortical bone toughness after three years of treatment in dogs, this was not statistically different than placebo-treated animals [10]. Measurements of femoral diaphyseal toughness in the canine model did not demonstrate a significant decline at either clinical or higher doses of ALN after three years of treatment [15]. However, cyclic fatigue loading of ribs from dogs treated for three years with ALN at the high dose resulted in a statistically significant 3-fold reduction in fatigue life compared to placebo-treated controls, suggesting reduced toughness. In this cyclic loading study, there was a significant trend for a dose effect detected by ANCOVA, but no significant difference was detected between the clinical dose of ALN and controls on a pairwise comparison [16].

Given that long-term treatment with BPs, on the order of five years or more, has been associated with serious side effects such as atypical femoral fractures [2,3], which occur in cortical bone, it is important to determine whether the intrinsic material properties of cortical bone, in particular toughness, are significantly impaired by BP treatment at clinical doses. The failure to demonstrate statistical significance using clinical doses of ALN even with relatively large declines in toughness of cortical bone in some studies suggests that the

sample sizes used in most large animal studies are not adequately powered to clearly identify effects on toughness. The goal of this work was to examine collectively cortical bone data from several of our previous studies, including new unpublished data from recent mechanical tests of dogs that were treated with ALN at clinical doses for 3, 6, 12 or 24 months. We hypothesized that there would be a significant reduction in toughness that was time-dependent.

Methods

All studies described herein utilized skeletally mature female beagles that began treatment around 1 year of age. Animals were part of 5 separate experiments that differed only by duration with the treatment phase lasting 3 months, 6 months, 1 year, 2 years, or 3 years. While data from several of these experiments have been previously published [6,8,10,17], rib toughness data have only been reported for the 3 year animals [10]. The Indiana University School of Medicine Animal Care and Use Committee approved all experiments prior to initiation of each study.

In each experiment, animals were treated with either saline as a control (CON, 1 ml/kg body weight) or alendronate (ALN, 0.2 mg/kg body weight) via a daily oral dosing regimen. The ALN dose was chosen to approximate the clinical therapeutic dose for osteoporosis on a mg/kg basis. At the conclusion of the treatment duration animals were euthanized with sodium pentobarbital. Ribs were excised, wrapped in saline soaked gauze, and stored at −20C until use.

Prior to mechanical testing, ribs were thawed to room temperature, cut to ~40 mm in length with the mid-point falling near the point of greatest curvature. The mid-point was marked and bone diameter in the AP direction at this site measured using digital calipers. Bones from 3 month, 6 month, and 3 year experiments were subjected to pQCT prior to mechanical testing. A single slice was taken at the point of testing using a Stratec Research M scanner at the maximal resolution (70 microns). Ribs from animals treated for 1 or 2 years were imaged with microCT after completion of mechanical testing. A region of interest centered at the point of failure was scanned using a Skyscan 1172 system at a resolution of 12 microns. A single slice, centered within this region of interest was reconstructed and analyzed. For both pQCT and microCT, the average cross-sectional moment of inertia (CSMI) of each individual dog was used to normalize mechanical test data to derive material properties.

All ribs were subjected to 3-point bending to determine their mechanical properties. Bones were thawed to room temperature and hydration was assured by soaking in PBS prior to testing. Mechanical tests were conducted on a servo-hydraulic test system (Test Resources for the 3 month, 6 month, 1 year, and 2 year experiments; MTS System for the 3 year experiment). For all tests, bones were placed on the support set up with the convex surface of the rib facing upward on a 25 mm bottom support span. Tests were conducted at a displacement rate of 20 mm/min with data collection of load vs displacement at 20Hz. Whole bone mechanical properties were determined by analysis of the load-displacement curve. Toughness (*u)* was estimated by normalizing energy absorption to fracture (as well as

pre- and post-yield) using the standard equation of u = 0.75 * energy absorption * (diameter^2/(span length * CSMI). The yield point was determined using the 2% offset criterion.

Statistical tests were performed on toughness data using ANOVA followed by pairwise comparisons at each time point. Linear regressions of percent difference between CON and ALN were run to assess time trends using intercept and slope values. For all tests, a two-sided p value of ≤ 0.05 was considered as statistically significant.

Results

Toughness, a measure of the intrinsic ability of the material to resist fracture, was lower in animals treated with ALN for at least a year, with borderline significance at the 3-year time point (−19%; p=0.06) (Figure 1A). Separation of toughness into pre-yield and post-yield components revealed similar results for post-yield toughness (3 year difference of 19%; p=0.06) with no differences in pre-yield toughness (Figure 1B and 1C).

Due to variability in CON animals across time points, possibly due to age-related effects, the influence of ALN was evaluated by determining percent difference between CON and ALN within time point. Linear regressions revealed a significant downward slope in toughness over time (y = −8.43x + 6.98; p = 0.042, Figure 2). The intercept was not significantly different than 0 (p = 0.23). The downward trend was similarly evident, although not statistically significant for post-yield toughness (slope p value = 0.13; intercept p value = 0.60) while there was no observed trend for pre-yield toughness over time (slope p value = 0.88; intercept p value = 0.84).

Discussion

These new data and analyses suggest that longer duration ALN treatment, at doses equivalent on a mg/kg basis to those used in the treatment of post-menopausal osteoporosis, will result in deterioration of cortical bone toughness in a time-dependent manner. Prior to one year of treatment there is a trend toward improved toughness, probably as a function of reduced remodeling that increases bone mineral density by up to 14% [4]. However, by one year of treatment, toughness is non-significantly lower than control and this difference remains through 3 years of treatment. There is a significant trend toward reduction in the energy absorbing capacity of cortical bone tissue between 1 and 3 years of treatment. In association with a suppression of remodeling that suppresses the repair of microdamage [4,10], this could lead to a downward spiral resulting in a bone fracture that has the appearance of a stress fracture. Moreover, extrapolated out to 5–7 years of treatment, the average duration of treatment for those patients on ALN who present with an atypical femoral fracture (AFF), these data suggest that long-term continuous treatment could be associated with a reduction in toughness well beyond 20% relative to an untreated control population. Although it is impossible to determine whether the monotonic decline in toughness would continue as it does in the first three years, this trend with clinical dosing regimens should nevertheless be a cause for concern.

The reduced bone toughness appears to occur almost exclusively in the post-yield portion of the stress-strain curve. Pre-yield toughness is largely determined by the elastic properties of the bone – its strength and stiffness – which are known to be improved with alendronate treatment. In this region, when the load on the bone is released, there is no permanent damage and the bone recovers its original properties (with some loss of energy). Toughness measured after the yield point - post-yield toughness - is a measure of the bone's capacity to absorb energy after permanent damage has occurred from microcrack formation. Once this point has been reached, the bone is not able to recover its original properties without a period of time for remodeling and repair. A smaller period of post-yield deformation results in lower toughness and can be a sign of increasing brittleness of the bone tissue.

Reduced post-yield toughness is consistent with previous data that indicates that cortical bone ultimate stress and modulus, pre-yield measurements of bone's intrinsic mechanical properties, are not affected significantly by ALN treatment [4,10,14]. The localization of reduced toughness to the post-yield portion of the stress-strain curve clearly implicates increased tissue brittleness as a cause of the impaired properties. This observation may be important given the fractography of atypical femoral fractures (AFF). These fractures are unusual in that they are not oblique, as femoral osteoporotic fractures typically are, but rather progress transversely across the femoral cortex, more typical of a brittle-type fracture.

Although the risk for and incidence of AFFs increases markedly with longer duration bisphosphonate treatment [18,19] the results of our current analysis showing a significant decline in toughness over time begs the question about why the clinical situation is not worse for patients who have been on alendronate for a long period of time. One answer to this may be that the increased BMD and preserved architecture associated with alendronate treatment compensates, in some individuals or for some period of time, at the structural level for the negative effects of alendronate on material properties. It is also possible that suppression of the normally high rate of bone turnover in the rib accelerates the process of material degradation, although even greater reductions in toughness were found in the vertebral trabecular bone in the same animals despite slower remodeling rates [8].

It is not possible to determine whether similar effects on tissue properties would be caused by treatment at clinical doses using other BPs. Pre-clinical data on dogs using risedronate at clinical doses [4,6,13] have only used experimental treatment periods up to one year. Pre-clinical data using incadronate in dogs did not use a clinically-relevant dose [14]. Pre-clinical studies using other large animal models typically have not measured changes in cortical bone toughness [9,20] and so would not have been able to detect this negative trend on bone's energy absorption capacity. Our own studies on dog femoral beams did not show an effect of ALN treatment on toughness [15].

It is difficult to identify long-term problems that may occur with any pharmaceutical treatment, in part because it is too expensive to perform long-term studies with large animals. The use of large animals is particularly important for cortical bone assessment, as rodents do not typically undergo intracortical remodeling. As a key effect of bisphosphonates is to reduce remodeling, the cortical bone response to bisphosphonates will be fundamentally different in rodents and larger species. Because of the high cost and strict

federal regulation of large animals, these studies are often conducted with the smallest sample size within each time cohort that can provide adequate statistical power. Statistical power, as in this case, is often calculated based on outcome measures that are expected to change with treatment (eg BMD). However, when an important variable, such as toughness, unexpectedly changes as in our studies, any single study may not be sufficiently powered to unequivocally demonstrate the significance of the change in that parameter. In these cases, analyzing data from several studies collectively, as we have done here, can be a powerful means to demonstrate consistent and significant changes with duration of treatment.

One limitation of this work is that the dogs were not ovariectomized, and thus do not represent an estrogen-deficient state as would be present in patients treated with bisphosphonates for post-menopausal osteoporosis. With low levels of circulating estrogen, most studies have shown that ovariectomy has minimal effect on the dog skeleton with respect to increasing remodeling rate and decreasing bone mass [21,22]. It is also important to note that since the data presented here is a compilation of experiments conducted over nearly 10 years, some of the methods (specifically imaging to determine cortical bone geometry) were different among studies. The methods were internally consistent within time point (control and treated animals were assessed similarly) making the comparisons valid. Finally, it would have been desirable to use more sophisticated statistical methodology. However, the statistical approach was limited because (a) each animal is represented by only a single data point so we cannot account for baseline values or within subject variations; (b) there are too few time points to allow for more complex models that could evaluate trends; and (c) the animals were rather homogeneous in terms of sex (female), species (beagle), and age (1–4 years old). This prevents the evaluation of covariate effects. Therefore, a linear model including ANOVA is the appropriate approach.

In conclusion, these new data suggest that alendronate treatment at clinical doses is associated with a significant time-dependent decline in cortical bone energy absorption capacity. Changes occur in the post-yield region of the loading curve, suggesting that long duration bisphosphonate treatment is associated with brittle behavior of the cortical bone. Given the recent increase in incidence of atypical femoral fractures, and the future projected increase with longer-term treatments [18], this should be a significant cause for concern.

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Highlights

- Longer treatment with clinical doses of alendronate in dogs reduces cortical bone toughness in a time-dependent manner
- As treatment period is prolonged, cortical bone exhibits increasingly brittle behavior
- This is relevant to assessing effects of long-term alendronate therapy on the risk of atypical femoral fractures in humans

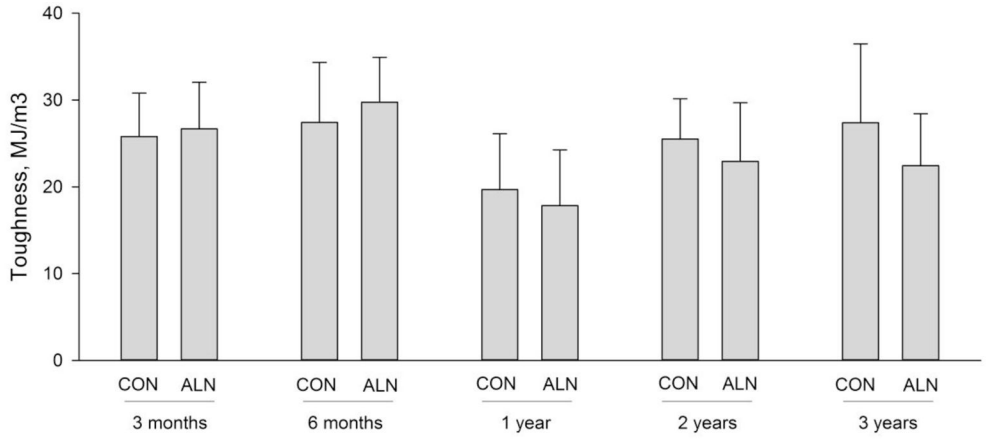

Figure 1a

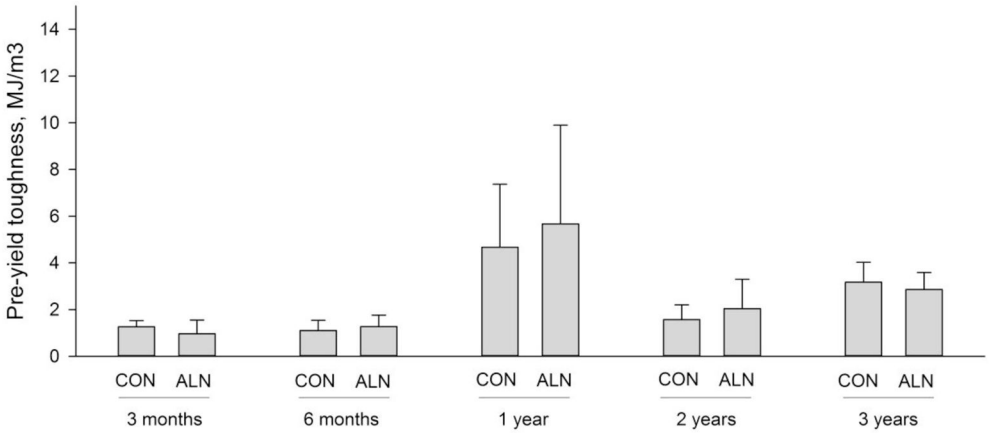

Figure 1b

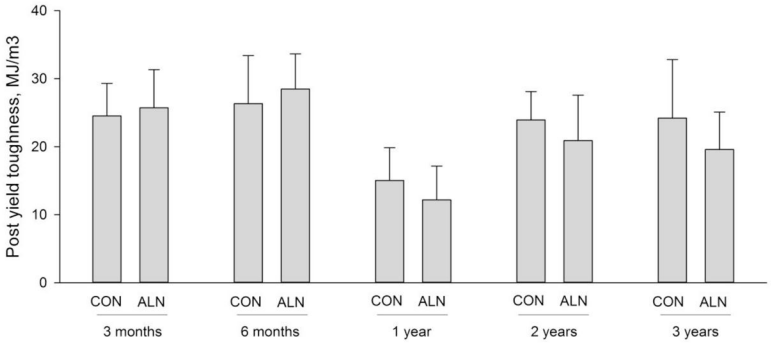

Figure 1c

Figure 1.

Toughness of rib cortical bone following 3 months to 3 years of treatment with oral alendronate (0.2 mg/kg/day orally) compared to control animals. (A) Toughness, (B) Pre-yield toughness, and (C) post-yield toughness. Data presented as mean and standard deviations.

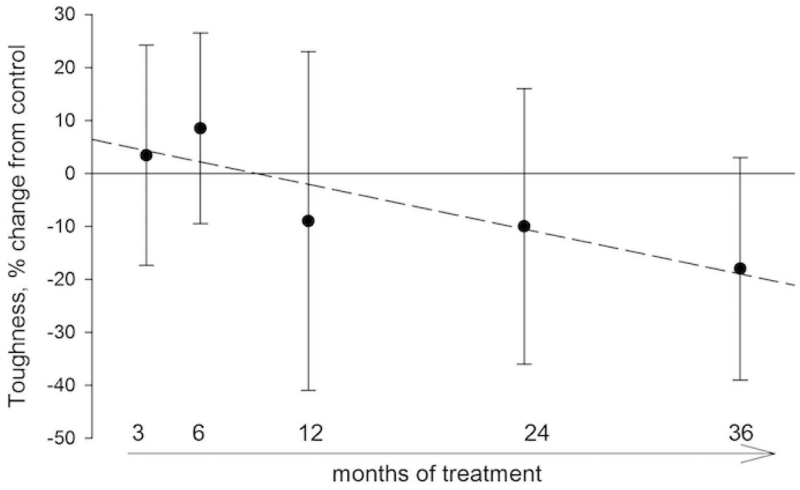

Figure 2.

Linear regression of alendronate's effect over time relative to control animals showed a significant duration-dependent decline in toughness ($p = 0.042$). Data presented as mean and standard deviations.