One Year of Alendronate Treatment Lowers Microstructural Stresses Associated with Trabecular Microdamage Initiation

+O’Neal, Jessica¹,²; Diab, Tamin¹,²; Allen, Matthew¹; Burr, David¹; Guldberg, Robert¹
+²Georgia Tech, Atlanta, GA, ³Medical College of Georgia, Augusta, GA, ⁴Indiana University School of Medicine, Indianapolis, IN
Senior author: robert.guldberg@nc.gatech.edu

INTRODUCTION:
Long-term alendronate treatment has been shown to reduce bone toughness in vertebral bone of beagle dogs¹, but the effects of bisphosphonates on stresses and strains within microdamaged trabeculae have yet to be reported. This study modifies a microdamage classification system published previously² to correlate trabecular stresses and strains to test-induced microdamage severity, and evaluates alendronate’s effect on these trabecular mechanical properties.

METHODS:
Cores from the distal femur of beagle dogs treated for one year with alendronate (0.2 mg/kg/day, post-menopausal osteoporosis treatment dose; n=3; or 1.0 mg/kg/day, Paget’s disease treatment dose; n=3) or a saline vehicle (1.0 mL/kg/day; n=3) were imaged with micro-computed tomography (µ-CT, voxel resolution of 20 µm) and mechanically tested in uniaxial compression to the yield strain (1.2%). Prior to mechanical testing, cores were stained with 0.02% Alizarin ComplexOne to label pre-existing microdamage. After testing, samples were stained with 0.01% Calcein to label test-induced microdamage. Samples were embedded in methyl-methacrylate (MMA), cut on a diamond saw to a thickness of ~150 µm, and mounted onto slides.

Values for bone mineral density were obtained by thresholding and evaluating µ-CT images. Finite element models (FEM) of principal compressive and tensile stresses and strains as well as Von Mises stresses and strains were created from µ-CT images. Test-induced trabecular damage was first identified under green epifluorescence then divided into 7 categories based on a published classification system.² Four gross damage categories were created by combining damage from the previous classification system into severely damaged, linear damaged, diffuse damaged, and undamaged groups. The histological slides obtained from cutting mechanically tested bone cores were visually registered to the µ-CT images, and mechanical parameters of individual trabeculae (n=300) were obtained from the FEM output.

One-way ANOVA tests and Tukey pairwise comparisons were conducted to assess statistical significance between mechanical parameters, damage state, and treatment effect. T-tests were used to assess statistical significance between damaged and undamaged states.

RESULTS:
The bone volume fraction (BVF) of each sample was calculated using automated distance transformation algorithms on µ-CT images. The average BVF of the control group (0.12 ± 0.022) was significantly lower than the BVF of the 0.2 mg/kg/day group (0.22 ± 0.049, p=0.03), but not significantly different from the BVF of the 1.0 mg/kg/day group (0.18 ± 0.03). The tissue modulus of each sample was back-calculated under an apparent modulus obtained during mechanical testing. The average tissue moduli for the control group (15.3 ± 2.4 GPa), the 0.2 mg/kg/day dose group (10.7 ± 2.5 GPa), and the 1.0 mg/kg/day dose group (14.4 ± 4.4 GPa) were not significantly different.

Analysis of trabecular principal compressive strains revealed significant differences in the local strain values associated with damage type (Figure 1a). Severely damaged trabecular strains were greater than linear damage, diffuse damage, and undamaged trabeculae. Linear and diffuse damaged trabecular strains were significantly greater than undamaged trabeculae. Additionally, significant differences between damaged and undamaged trabeculae within treatment groups were seen (Figure 1b). Results for von Mises strains were similar.

Analysis of trabecular principal compressive stresses showed significant increases in severely damaged trabeculae compared to other damage types (Figure 1c), though differences between linear, diffuse, and undamaged trabecular stresses were not significant. Results for von Mises stresses were similar.

Samples treated with 0.2 mg/kg/day alendronate showed significantly lower principal compressive and Von Mises stresses compared to control and 1.0 mg/kg/day doses (Figure 1d), with severely damaged trabecular damaging at stresses significantly lower than that of other treatment groups. Undamaged trabeculae were also at significantly lower stress states in the 0.2 mg/kg/day dose than the 1.0 mg/kg/day dose. No other treatment effects were noted.

DISCUSSION:
This study analyzed the effect of alendronate on the stress and strain state of trabeculae in various states of damage. A microdamage classification system was used in which differences in stress and strain magnitude correlated with damage severity.

The results can be viewed in the context of a range of strains or stresses at which microdamage initiates. From figures 1b and 1d, strain and stress initiation ranges for each treatment group are computed: control=0.55-0.91% strain, 86.9-111.9 MPa; 0.2 mg/kg/day dose=0.61-0.92% strain, 64.4-84.4 MPa; and 1.0 mg/kg/day dose=0.66-0.89% strain, no distinct stress range. Thus, one-year of alendronate treatment has no significant effect on the strain, but significantly lowers stresses needed to induce microdamage. We speculate that this effect was not observed in the high dose of alendronate because the tissue modulus was no distinct strain range. Therefore, one-year of alendronate treatment dose of alendronate decreases the stress initiation range for microdamage formation. Future studies will investigate how microdamage initiation ranges change with alendronate use over time.


ACKNOWLEDGEMENTS: NIH R01 AG027249

Figure 1. a) Principal compressive strains are plotted by type of damage. Significant differences are noted between severely damaged (*p<0.001), linear damaged (**p=0.008), and diffuse damaged (***p=0.002) compared to undamaged trabeculae. Severely damaged trabeculae are also at a significantly higher strain than linear and diffuse damaged (p<0.001). b) Damaged vs. undamaged trabecular strains are plotted. All treatment groups show significant differences between damaged and undamaged trabeculae (p<0.001). c) Principal compressive stresses of trabeculae are plotted by type of damage. Severely damaged trabeculae are under higher stress states than linear (p<0.001), diffuse (p<0.001) and undamaged trabeculae (p<0.001). d) Principal compressive stresses are plotted by treatment group. The 0.2 mg/kg/day treatment dose is under significantly lower stresses in both the damaged and undamaged state compared to the other treatment groups (p<0.001). Error bars represent the population mean at 95% CI for all plots.