

Bone quality changes with Alendronate do not rescue fragility in brittle bones

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INTRODUCTION: There is currently no cure for osteogenesis imperfecta (OI or Brittle bone disease) a disorder of genes encoding collagen type I and characterized by skeletal fragility and bone deformities [1]. Management treatments for OI rely on the use of bisphosphonates, a group of antiresorptive drugs FDA approved for osteoporosis that are given to OI children with controversial results [3]. Since bone fracture is the primary symptom of OI, it is critical to understand how this drug affects OI bone quality properties and resistance to fracture. In this study, we investigate whether long-term alendronate (ALN) treatment effectively improves bone fracture toughness (i.e., resistance to fracture) in the *oim* mouse model of OI. For the purpose, a digital image correlation (DIC) system was used during loading and crack progression to characterize the mechanical environment of the periosteal bone surface around the crack. Finally, variations in bone mineralization and collagen fibers organization were studied using 3D Raman microspectroscopy and Second Harmonic Generation (SHG) microscopy, respectively.

METHODS: Femurs of 14-week-old alendronate-treated (ALN 0.21 mg/kg/week, 0.1 ml/1g BW starting at 2 weeks of age) and saline control (CTR) B6C3fe-a/col1a2*oim/oim* (*oim/oim*) and wild-type (WT) mice (N=5/group/sex) were notched, surface speckled, and tested in 3-point bending for fracture toughness (at 0.01 mm/s) while 2 CCD cameras (100 mm focal lenses) recorded images of the crack growth on the external bone surface at 22 Hz. Bone surface strains were calculated by Aramis SRX System (GOM) during the crack growth. Following the mechanical test, bone fracture surfaces were imaged in back-scattered mode in an ESEM (Zeiss Supra 55), and bone fracture toughness was calculated. The crack mouth opening displacement (CMOD), i.e. the change in distance between the two faces of the notch, was measured at different instances of the load using the DIC images. Afterwards, fractured bones were embedded in polymethyl methacrylate, and blocks were sliced and polished exposing the stable crack growth site. 3D Raman microspectrography was acquired using a WiTec Confocal Raman microscope alpha300R, with a 633 nm monochromatic laser emitting at 18 mV of power, using a 50X magnification and a spectral resolution of 3 cm⁻¹. Point-by-point stacks of bone area were scanned across the crack path and volumetric chemical maps were reconstructed across the depth of the tissue. 3D spatial distribution of mineral-to-matrix ratio, crystallinity and carbonate-to-phosphate ratio were obtained using Project 5 software and a custom MATLAB code. SHG microscopy images were acquired using a Prairie Technologies Ultima IV Multiphoton Microscope (Bruker) at an excitation wavelength of 920 nm using a 40X magnification and 0.8 N.A. water immersion objective lenses. Quantitative analysis of collagen amount, orientation and organization was performed using the new FiberO software developed in our lab [4]. Statistical analysis was conducted to discern significant differences between genotypes, treatment and sex.

RESULTS SECTION: *Oim/oim* bones exhibited a drastic decrease in fracture toughness compared to WT bones. Long-term ALN treatment did not change *oim/oim* and WT value of bone fracture resistance. The CMOD was smaller in *oim/oim* bones vs. WT ones, both with and without treatment. Only WT bones (both controls and ALN treated) had similar Load-CMOD curves and exhibited crack deflections. ALN treated *oim/oim* bones had 40% shorter CMOD, 43% higher maximum normalized load and twice the slope of the *oim/oim* sham bones. 3D Raman microspectrography of the fracture sites showed increased mineralization (24%) and decreased carbonate substitution (11%) in *oim/oim* bones compared to WT. ALN treatment increased carbonate substitution in both *oim/oim* and WT bones (3% and 8%, respectively), and decreased mineralization only in WT bones (30%) compared to their respective sham controls. The analysis of SHG microscopy images revealed a decrease in the collagen amount and percentage of organized fibers in the *oim/oim* bones, and an increase in the quantity of organized collagen fibers as a result of ALN administration. No sexual dimorphisms were observed.

DISCUSSION: In this work we present evidence that long-term ALN-treatment does not rescue *oim* bone fragility in *oim/oim* bones, although the mechanism to resist fracture differs in ALN-treated *oim/oim* bones: the fibers are slightly more organized and mineralized and can support higher loads with an increased stiffness, but catastrophically break with very little crack mouth opening. Our future studies will look at the effect of short-term ALN on *oim/oim* bone fracture resistance.

SIGNIFICANCE/CLINICAL RELEVANCE: This study gives insight into the actual effectiveness of bisphosphonates for the management of bone fragility in young OI population. This work provides evidence that skeletal fragility remains a problem in OI bone treated with bisphosphonates, and thus new treatment strategies are needed to improve fracture resistance in brittle bone disease.

REFERENCES:

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