INTRODUCTION

Osteogenesis Imperfecta (OI) is a genetic disease of collagen or collagen-related proteins that adversely impacts bone mass and fragility. Little is known regarding the role that microdamage (µ.Dx) plays in OI and whether or not OI bone is more prone to damage accumulation than bone with unaffected collagen. The Brtl/+ mouse is a heterozygous model for OI. The genotype arises from a Gly349Cys substitution in COL1A1, and demonstrates a low ductility phenotype [1]. Brtl/+ also demonstrates an increase in osteoclast number and activity at 8 weeks of age, mimicking the upregulated bone turnover often found in OI [2]. We hypothesize that upregulated osteoclast activity in Brtl/+ is due, in part, to increased remodeling associated with microdamage repair. We also suspect that the increased rates of fracture seen in OI patients, is correlated with an increase in the quantity of microdamage present in these patients through every day ambulation. In the present study, we use Brtl/+ to investigate the susceptibility of OI bone to microdamage and fracture. The mouse ulnar loading model is used to induce microdamage and to test the hypothesis that Brtl/+ is more susceptible to damage accumulation than age-matched wild type (WT) counterparts.

MATERIALS AND METHODS

Mice: A total of 18 Brtl/+ and WT mice (n=9/group) were used in this study. Brtl/+ mice have a mixed background of Sv129/CD-1/C57BL/6S and are bred by crossing heterozygous Brtl/+ with WT. At 8 weeks of age, mice were euthanized for subsequent loading experiments.

Cyclic Loading: Strain gauges were placed in-situ on the right ulnae of Brtl/+ (n=3) and WT (n=3) mice at the mid-diaphyses. Forearms were then cyclically loaded in an axial direction using a custom ulnar loading device to obtain the load-strain relationship for both genotypes, as differences in limb size between Brtl/+ and WT have been shown². Compressive strains approaching 4000 µε have been shown to induce microdamage[3]. Therefore, the forces that would induce 4000 µε in Brtl/+ and WT were ascertained for subsequent cyclic loading experiments (2.7 N for Brtl/+ and 4.9 N for WT). The right forearms of Brtl/+ (n=6) and WT (n=6) were then cyclically loaded to these maximum strain values at 1 Hz for 1800 cycles (30 minutes) following a 300 cycle (5 minute) preconditioning phase. During the unloading segment, a minimum compressive load of approximately 400-500 µε was maintained to preserve limb orientation within the loading device. Loss of stiffness for each bone was determined using previously described techniques [4].

Microdamage Analysis: Bones were then dissected, stained with 1% basic fuchsin, embedded in PMMA and then sectioned and polished to 100 µm thickness. Slides were viewed using a confocal microscope (Carl Zeiss LSM 510-META) in order to quantify the degree of linear microdamage throughout the 100 µm thickness of each section.

Fracture Toughness: The same 8 week male WT and Brtl/+ mice (n=6/group) used in µ.Dx experiments were utilized. Left femora were harvested and cleaned of soft tissue. Femurs were machine notched on the posterior wall to a depth of 1/3 of the bone diameter. Notches were subsequently sharpened with a 1 µm diamond coated razor blade to produce a 10 µm diameter root notch. Femurs were loaded to failure using a 3-point test configuration at 0.001 mm/s rate. Following fracture, the femurs were defatted and dehydrated, and then carbon coated [5]. Notch and instability regions on the fracture surface were determined from Scanning Electron Microscope (SEM) images. Fracture toughness values were measured using solutions for circumferential through-wall cracks established for cylindrical pipes. Both the maximum load and crack instability methods were used in this study [7]. Due to premature fracture during 3-point bend setup and errors in the notching process, a sample of n=4/group was eventually assessed for fracture toughness analysis.

Statistics: Effect of genotype on percent stiffness loss, crack numerical density, crack surface density, and fracture toughness were assessed by t-test with significance attributed to p<0.05. Data is presented as mean±SD.

RESULTS AND DISCUSSION

In unloaded control limbs, Brtl/+ ulnae displayed greater crack numerical density (117%, Figure 1A) and crack surface density (254%, Figure 1B) compared to age-matched WT controls. Over the 35 minute loading cycle, Brtl/+ ulnae displayed a lower loss of stiffness (1.77%) compared to WT (3.56%) (Figure 2). However, despite this lower stiffness loss, Brtl/+ limbs were more prone to the accumulation of damage compared to WT (Figure 1). Both crack numerical density and
surface density were increased in loaded Brtl/+ limbs compared to WT controls (120% and 231% respectively, p<0.05). Brtl/+ fracture toughness using the maximum load and crack instability techniques was reduced compared to WT (18.6% and 31.4% respectively, Figure 3), although low sample size prevented these reductions from achieving statistical significance (p=0.39; 0.11 respectively).

Brtl/+ ulnae subject to normal cage activity demonstrate an inherently larger amount of microdamage than WT controls. Furthermore, following 35 minutes of axial compressive loading, Brtl/+ ulnae are more prone to damage than WT counterparts despite demonstrating a greater resistance to whole-bone deformation. This increased stiffness is likely due to increases in cortical tissue mineral density associated with the collagen mutation [6].

CONCLUSIONS
Increased microdamage and reduced fracture toughness observed in the murine model for osteogenesis imperfecta suggests that microdamage may play a significant role in contributing to bone fragility associated with OI. Furthermore, at 8 weeks of age, Brtl/+ have increased osteoclast number and activity [2]. This increased remodeling may be due, at least in part, to an increase in targeted microdamage repair. These findings may have strong clinical implications for explaining increased fragility and remodeling activity in OI patients.


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