Sclerostin Antibody Improves Skeletal Parameters in Brtl/+ Model of Osteogenesis Imperfecta

INTRODUCTION
Osteogenesis imperfecta (OI) is a genetic bone disorder characterized by brittle bones with high propensity to fracture. Presently, bisphosphonates are widely used to treat children who have OI. A new bone anabolic agent, sclerostin antibody (SclAb), may be beneficial for treatment of OI, where reduced rates of osteoblast activity have been identified. The anabolic nature of the SclAb may be beneficial in reversing this phenotype of reduced bone formation, thus increasing bone mass and reducing fracture risk.

The purpose of this study was to investigate short-term SclAb treatment in Brtl/+ mice, a well characterized model for OI. We demonstrate that SclAb therapy increases trabecular and cortical microCT parameters via anabolic mechanisms which increase the mineralizing surface of osteoblasts in Brtl/+ OI bone.

METHODS
8 week male WT and Brtl/+ mice were randomly assigned to SclAb treatment (Brtl/+ n=5; WT n=2) or vehicle (PBS) groups (Brtl/+ n=4; WT n=4). SclAb (25mg/kg twice per week, Amgen) was administered by s.c. injection for two weeks. Calcein (30mg/kg) i.p. injections were performed at the start of the experiment, after 1 week, and 1 day prior to sacrifice to monitor changes in bone formation and identify regions of bone formed during the treatment period. The study was approved by the University Committee on Use and Care of Animals.

Left femora were imaged by microCT (GE eXplore Locus SP) at 18 µm isotropic voxel size. Both mid-diaphyseal cortical bone and distal trabecular bone regions of interest were analyzed. After microCT images were acquired, left femora were mechanically tested to failure in four point bending with the posterior surface of the mid diaphysis in tension using a servohydraulic testing system (MTS 858 MiniBionix).

Right femora were encased in a fast curing PMMA (Koldmount) for dynamic histomorphometry and nanoindentation. Each specimen was sectioned and polished to a mid diaphyseal region where mineralizing surface, bone surface, and mineral apposition rate were quantified on the periosteal surface for the two weeks of treatment (Bioquant Osteo).

Nanoindentation was subsequently performed on the same right femora to assess changes in bone elastic modulus. A custom nanoindenter (Hysitron Triboindenter) equipped with a fluorescent microscope was used to visualize calcein labeling, allowing indent location to be controlled for by tissue age and to precisely locate indents in regions of bone formed during the treatment period.

All comparisons between treated and untreated animals within genotype were assessed by Student’s t-test, with p<0.05 considered significant (*).

RESULTS
In the trabecular compartment of the distal femur, SclAb treatment significantly increased bone volume fraction in Brtl/+ and WT animals (data not shown). This was attributable to significant increases in trabecular thickness (Fig 1A) and trabecular bone formation rate (Fig 1B) in Brtl/+.

In cortical bone, SclAb treatment significantly increased osteoblast bone formation rate in both Brtl/+ and WT femoral diaphyses (Fig 2C). Untreated Brtl/+ femora showed triple calcein labeling primarily at the lateral cortex while SclAb induced new bone formation around the entire periosteal surface. SclAb had no effect on Brtl/+ osteoblast mineral apposition rate (Fig 2A), and increased BFR through an 86% increase in mineralizing surface (Fig 2B). WT bone formation rate was dominantly increased through an 80% increase in MAR (Fig 2A), with no corresponding increase in mineralizing surface (Fig 2B).

These increases in bone formation conferred improved geometrical properties as measured by microCT (Fig 3A). As a result, these improved geometrical properties contributed to an increased ultimate load as measured by mechanical testing of the left femora (Fig 3B). Although not significant, after only two weeks of treatment, ultimate load, stiffness, and energy were all increased in Brtl/+ with treatment. Nanoindentation within the cortex and at each calcein label reveals a progressively lower elastic modulus with decreasing tissue age.

DISCUSSION
Two weeks of SclAb treatment significantly increased cortical and trabecular bone parameters in both WT and Brtl/+ animals. While alendronate previously only increased trabecular bone in Brtl/+ after 6 weeks of treatment, trabecular thickness was also improved in the present study. Osteoblast deficiencies in Brtl/+ were rescued, and bone mass was improved. While improvements in bone strength were not significant with two weeks of treatment, longer term treatment may suggest SclAb can reduce propensity to fracture. These initial data support that sclerostin antibody may be beneficial for treatment of OI patients by stimulating the deficient osteoblast population.

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