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Rapidly growing Brtl/+ mouse model of osteogenesis imperfecta improves bone mass and strength with sclerostin antibody treatment

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ABSTRACT

Osteogenesis imperfecta (OI) is a heritable collagen-related bone dysplasia, characterized by brittle bones with increased fracture risk that presents most severely in children. Anti-resorptive bisphosphonates are frequently used to treat pediatric OI and controlled clinical trials have shown that bisphosphonate therapy improves vertebral outcomes but has little benefit on long bone fracture rate. New treatments which increase bone mass throughout the pediatric OI skeleton would be beneficial. Sclerostin antibody (Scl-Ab) is a potential candidate anabolic therapy for pediatric OI and functions by stimulating osteoblastic bone formation via the canonical Wnt signaling pathway. To explore the effect of Scl-Ab on the rapidly growing OI skeleton, we treated rapidly growing 3 week old Brtl/+ mice, harboring a typical heterozygous OI-causing Gly → Cys substitution on *col1a1*, for 5 weeks with Scl-Ab. Scl-Ab had anabolic effects in Brtl/+ and led to new cortical bone formation and increased cortical bone mass. This anabolic action resulted in improved mechanical strength to WT Veh levels without altering the underlying brittle nature of the material. While Scl-Ab was anabolic in trabecular bone of the distal femur in both genotypes, the effect was less strong in these rapidly growing Brtl/+ mice compared to WT. In conclusion, Scl-Ab was able to stimulate bone formation in a rapidly growing Brtl/+ murine model of OI, and represents a potential new therapy to improve bone mass and reduce fracture risk in pediatric OI.

1. Introduction

Osteogenesis imperfecta (OI) is a heritable collagen-related dysplasia that results in bone fragility [1]. This condition presents with a wide range of clinical severity, depending upon the type of OI and specific mutation. While OI persists throughout life, the symptoms and fracture risk are greatest during childhood [2].

Numerous therapies have been tried to reduce fracture risk in pediatric OI. The most widely used treatment is anti-resorptive bisphosphonates. Multiple clinical trials with pediatric OI and bisphosphonates have demonstrated their efficacy at increasing vertebral BMD and surrogate measures of vertebral strength; however, the effect on long bone fracture risk appears equivocal [3–8]. Treatment of pediatric OI with growth hormone (rGH) has shown encouraging results for an anabolic therapy, but was not consistently effective among all patients [9]. In positive responders, bone histology and mass were improved, and long bone fracture rates decreased, along with increased linear growth in some mildly and moderately severe pediatric OI patients [9,10]. Intermittent PTH is not used in children before growth plate fusion because of a reported osteosarcoma risk in rats, and as a result is not available as a treatment for pediatric OI [11].

Neutralizing antibodies to sclerostin are a candidate anabolic therapy for children with OI. Sclerostin is a potent inhibitor of bone formation which is secreted primarily by osteocytes [12]. Broadly, sclerostin functions by inhibiting canonical Wnt signaling through its binding to the Wnt signaling co-receptors LRP5 and LRP6 present on cells of the osteoblast lineage [13]. Neutralizing antibodies have been developed which functionally reduce sclerostin activity and thereby prevent sclerostin inhibition of bone formation. An anabolic response to sclerostin antibodies has been demonstrated in numerous pre-clinical studies [14–17] as well as phase I and phase II trials of osteoporosis [18,19].

The Brtl/+ mouse is heterozygous for a G349C mutation on col1a1 found in an OI patient, and is a model for moderately severe Type IV OI [20]. Brtl/+ recapitulates many of the phenotypic features of pediatric OI including small size, impaired remodeling, reduced trabecular and cortical bone mass, altered bone matrix structure, and impaired fracture mechanics [20–23]. As such, Brtl/+ has been used to explore multiple

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clinically relevant questions including fracture repair [24], bisphosphonate treatment [25], and cell therapies [26].

Treatment of the rapidly growing skeleton of children with OI is the most clinically relevant time point. Previously, we reported that a short-term, 2 week treatment of Brtl/+ with Scl-Ab successfully induced an anabolic response in 8 week old Brtl/+ mice [27]. While this proof-of-concept study demonstrated anabolic efficacy, it was not a direct correlate to the pediatric clinical condition, as mice only gained 2–5% body mass over the 2 weeks of treatment. In contrast, 3 week old Brtl/+ mice in the present study more than doubled their body mass over the 5 week treatment duration, highlighting the large difference between these two ages. To directly address whether Scl-Ab can elicit an anabolic response in an OI model during periods of rapid bone growth and body mass accrual, we treated rapidly growing 3 week old WT and Brtl/+ mice for 5 weeks with Scl-Ab and hypothesized that therapy would lead to increased bone mass and strength by stimulating bone formation.

2. Materials and methods

2.1. Animals

Wildtype (WT) and Brtl/+ [20] mice are maintained on a mixed background of Sv129/CD-1/C57BL/6S, and all Brtl/+ animals were the product of breeding male heterozygous Brtl/+ with female WT. 3 week old male WT and Brtl/+ mice were randomly assigned to Scl-Ab (Scl-Ab VI, Amgen, Thousand Oaks, CA) treatment or vehicle injection (PBS) with WT Veh n = 9, WT Scl-Ab n = 9, Brtl/+ Veh n = 8, and Brtl Scl-Ab n = 9. Sclerostin antibody was injected subcutaneously at 25 mg/kg, two times per week, following our previously described protocol [27,28], for five weeks. Calcein (30 mg/kg, i.p. injection) was injected at the start of the experiment (3 weeks animal age), after 2 weeks of treatment (5 weeks animal age), and after 4 weeks of treatment (7 weeks animals age). A final alizarin label (30 mg/kg, i.p. injection) was given 1 day before euthanasia (7 weeks + 6 days of animal age). The multiple fluorescent labels were used to visualize the growth pattern during the entire course of therapy. Body weights were recorded with each injection. Blood samples were collected at sacrifice by intracardiac puncture, and serum was separated by centrifuge, and stored at −80 °C until analyzed by ELISA.

Left femurs were collected for microCT and mechanical testing, and right femurs for dynamic histomorphometry. Both were stored at −20 °C in lactated Ringers solution (LRS) soaked gauze until testing or further specimen preparation. All protocols and procedures involving animals were approved by the University of Michigan's Committee on Use and Care of Animals.

2.2. Bone length and growth

Left femur and tibia lengths were measured longitudinally using whole body radiographs taken at the beginning and end of experiments on Scl-Ab or Veh treated animals. At 3 weeks of age, animals were briefly anesthetized with isoflurane, and microradiographs were taken in the coronal plane at 3× magnification (Faxitron MX-20, Faxitron X-Ray LLC, Lincolnshire, IL). Limbs were immobilized with tape during imaging to minimize motion and keep the leg bones flat. At 8 weeks of age, the radiograph protocol was repeated immediately after sacrifice. A lead ruler was used for calibration of the field of view, and bone lengths were measured with calipers.

2.3. Serum assays

To measure osteoblast activity, serum osteocalcin (OCN) was quantified with a commercially available ELISA kit (BT-470, BTI, Stoughton, MA). As a measure of osteoclast number, serum TRACP5b was measured with a commercially available solid phase immunofixed enzyme activity assay (MouseTRAP, IDS, Fountain Hills, AZ). Both serum tests were performed in duplicate. In several animals, inadequate serum volume was available to perform the assay, thus resulting in a modest reduction in group size.

2.4. MicroCT

Left femora were scanned in water using cone beam computed tomography (eXplore Locus SP, GE Healthcare Pre-Clinical Imaging, London, ON, Canada). Scan parameters included a 0.5° increment angle, 4 frames averaged, an 80 kVp and 80 μA X-ray source with a 0.508 mm Al filter to reduce beam hardening artifacts, and a beam flattener around the specimen holder [29]. All images were reconstructed and calibrated at an 18 μm isotropic voxel size to a manufacturer-supplied phantom of air, water and hydroxyapatite. Regions of interest (ROI) were located for both cortical and trabecular parameters. A diaphyseal cortical ROI spanning 15% of total femur length was located midway between the distal growth plate and third trochanter. Cortical bone was isolated with a fixed threshold of 2000 Hounsfield Units for all experimental groups. Parameters including cortical thickness, cross sectional area, marrow area, total area, anterior–posterior bending moment of inertia and tissue mineral density (TMD) were quantified with commercially available software (MicroView v2.2 Advanced Bone Analysis Application, GE Healthcare Pre-Clinical Imaging, London, ON, Canada). A trabecular ROI 10% of total femur length was located immediately proximal to the distal femoral growth plate and defined along the inner cortical surface with a splining algorithm. Due to the different morphology induced by Scl-Ab treatment, a fixed threshold could not be utilized without bias. Trabecular metaphyseal bone was isolated with a more conservative autothresholding algorithm for each specimen based on the bimodal distribution between marrow and bone [30]. Parameters including bone volume fraction (BV/TV), trabecular thickness (Tb.Th), and trabecular number (Tb.N), and trabecular bone mineral density (Tb.BMD) were quantified using standard stereology algorithms (MicroView v2.2).

A qualitative difference in femoral trabecular bone morphology was noticed as a function of distance from the growth plate. To quantify this, the 10% of bone length region of interest was sub-divided into the 5% of bone length region that was more distal (closer to the growth plate), and the 5% region that was more proximal. The same threshold determined for the entire 10% of bone length ROI was used for each of the 5% of bone length subdivided regions. In this way, the relative contribution of each sub-region could be assessed.

2.5. Whole bone mechanical four-point bending

Following μCT scanning, left femora were loaded to failure in four-point bending using a servohydraulic testing machine (MTS 858 MiniBionix, Eden Prairie, MN). All specimens were kept hydrated in LRS-soaked gauze until mechanical testing. In the same mid-diaphyseal region analyzed by μCT, the femur was loaded in four-point bending with the posterior surface oriented under tension. The distance between the wide, upper supports was 6.26 mm, and the span between the narrow, lower supports was 2.085 mm. The vertical displacement rate of the four-point bending apparatus in the anterior–posterior direction was 0.5 mm/s. Force was recorded by a 50 lb load cell (Sensotec) and vertical displacement by an external linear variable differential transducer (LVDT, Lucas Schavitts, Hampton, VA), both at 2000 Hz. A custom MATLAB script was used to analyze the raw force-displacement data and calculate all four-point bending parameters. Combining anterior–posterior bending moment of inertia data from μCT with mechanical stiffness from four point bending, the estimated elastic modulus was calculated using standard beam theory as previously described [21].