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Effect of sclerostin antibody treatment in a mouse model of severe osteogenesis imperfecta ☆

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ABSTRACT

Osteogenesis imperfecta (OI) is a heritable bone fragility disorder that is usually caused by mutations affecting collagen type I production in osteoblasts. Stimulation of bone formation through sclerostin antibody treatment (Sost-ab) has shown promising results in mouse models of relatively mild OI. We assessed the effect of once-weekly intravenous Sost-ab injections for 4 weeks in male *Col1a1*^{Jrt}/+ mice, a model of severe dominant OI, starting either at 4 weeks (growing mice) or at 20 weeks (adult mice) of age. Sost-ab had no effect on weight or femur length. In OI mice, no significant treatment-associated differences in serum markers of bone formation (alkaline phosphatase activity, procollagen type I N-propeptide) or resorption (C-telopeptide of collagen type I) were found. Micro-CT analyses at the femur showed that Sost-ab treatment was associated with higher trabecular bone volume and higher cortical thickness in wild type mice at both ages and in growing OI mice, but not in adult OI mice. Three-point bending tests of the femur showed that in wild type but not in OI mice, Sost-ab was associated with higher ultimate load and work to failure. Quantitative backscattered electron imaging of the femur did not show any effect of Sost-ab on CaPeak (the most frequently occurring calcium concentration in the bone mineral density distribution), regardless of genotype, age or measurement location. Thus, Sost-ab had a larger effect in wild type than in *Col1a1*^{Jrt}/+ mice. Previous studies had found marked improvements of Sost-ab on bone mass and strength in an OI mouse model with a milder phenotype. Our data therefore suggest that Sost-ab is less effective in a more severely affected OI mouse model.

Introduction

Osteogenesis imperfecta (OI) is a heritable bone fragility disorder that is usually transmitted in an autosomal dominant fashion. In the large majority of cases, OI is caused by mutations in *COL1A1* and *COL1A2*, the genes encoding the collagen type I alpha chains [1]. Over 1500 different mutations in these genes have been identified. Such mutations can also give rise to extraskeletal manifestations, such as tooth abnormalities (dentinogenesis imperfecta), blue or gray sclera and hearing impairment [1].

Treatment with antiresorptive drugs from the class of bisphosphonates is the current standard of care for pediatric OI [2]. Through increases in bone mass and reductions in fracture rate, bisphosphonate therapy has improved the quality of life for OI patients. Nevertheless, current treatment strategies leave room for substantial improvements, as many children still suffer from frequent fractures and bone deformation despite bisphosphonate use.

The importance of sclerostin signaling for bone mass homeostasis is well recognized [3]. Sclerostin is a protein that is secreted by osteocytes and is thought to inhibit bone formation by interacting with LRP5 on the surface of osteoblasts [4]. Treatment with sclerostin antibodies (Sost-ab) stimulates bone formation and increases bone mass in animal models and in women with low bone density [5,6]. Sost-ab treatment has been proposed as a potential therapeutic approach for OI as well, as a significant increase in bone mass and in bone strength was observed in two knock-in mouse models of dominant OI (Brtl mouse, with a G349C mutation in *Col1a1* and a mouse harboring a G609C mutation in *Col1a2*) [7–9].

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Nevertheless, the response of OI bone to interventions such as Sost-ab may depend on the specific disease-causing mutation or on the severity of the phenotype. In the present study we assessed the effect of Sost-ab therapy in *Col1a1*^{Jrt}/+ mice, a mouse model of severe dominant OI that was generated through a mutagenesis screen and harbors a splice site mutation in exon 9 of the *Col1a1* gene [10].

Methods and materials

Mice

Col1a1^{Jrt}/+ mice with a T to C mutation within the splice donor site of exon 9 of *Col1a1* were used, as previously described [10]. The mutation leads to skipping of exon 9, causing an 18 amino acid deletion in the main triple helical domain of Col1a1. The mutation is symptomatic (spontaneous fractures, small body size) in the heterozygous state. *Col1a1*^{Jrt}/+ mice were bred on a FVB background. *Col1a1*^{Jrt}/+ mice were generously provided by Dr. J. Aubin, University of Toronto. Animals were housed in the Animal Care Facility of the Shriners Hospital Montreal. The project was approved by the McGill University Institutional Animal Care and Use Committee.

Male wild type (WT) and *Col1a1*^{Jrt}/+ mice were randomly assigned to Sost-ab treatment or control injections, starting at either 4 weeks (phase of rapid growth, 'pediatric model') or 20 weeks of age ('adult model') with n = 8 per group. Sost-ab (BPS804; developed in a collaboration between Novartis Inc and MorphoSys Inc) or control antibody was used. BPS804 had been isolated from the HuCAL GOLD® library using phage display technology [11,12]. It is a neutralizing human IgG2 lambda monoclonal antibody that binds both human and mouse sclerostin with high affinity. The control antibody was an unrelated antibody that had been raised against chicken lysozyme.

Antibodies were injected intravenously at a dose of 100 mg per kg body weight, based on prior studies by Novartis Inc. The total injection volume was 50 μL. Injections were given once per week over a period of 4 weeks. Mice were euthanized at the end of the 4-week intervention period, i.e., at the age of 8 weeks and at 24 weeks, respectively. To enable the analysis of dynamic histomorphometric measures, each mouse received two intraperitoneal injections of calcein (25 mg per kg body weight) at 5 days and at 2 days before sacrifice (8 weeks old) and at 6 days and at 2 days before sacrifice (24 weeks old).

Body weights were recorded at the time of each injection. Blood samples were collected at euthanasia by intracardiac puncture, and serum was separated by centrifuge and stored at −80 °C until analysis. Right femurs were collected for microcomputed tomography (microCT) and for mechanical testing. These were stored at −20 °C in phosphate buffered saline-soaked gauze until testing or further specimen preparation. Left femurs were collected for dynamic histomorphometry and for quantitative backscattered electron microscopy tests. Femur length was measured with a caliper. Lumbar vertebrae 2 to 4 were collected for histomorphometry.

Serum biochemistry

Serum levels of total calcium, inorganic phosphorus and alkaline phosphatase were determined by standard methods. Markers of bone formation (procollagen type I N-terminal propeptide, PINP; Mouse/Rat PINP, Immunodiagnostic Systems) and of bone resorption (C-telopeptide of collagen type I, CTX; RatLaps, Immunodiagnostic Systems) as well as serum sclerostin (Quantikine ELISA Mouse/Rat SOST, R&D) were quantified by enzyme immunoassays. Serum tests were performed in duplicate.

Bone histomorphometry

Histomorphometric analyses of trabecular bone were performed at the left distal femur (starting at 50 μm proximal to the growth plate to a distance of 1.4 mm from the growth plate) and at lumbar vertebra 4 (L4, entire trabecular compartment excluding a 50 μm band adjacent to each end plate). Specimens were fixed in 10% phosphate-buffered formalin, dehydrated in increasing concentrations of ethanol and embedded in methylmethacrylate. Undecalcified 6 μm thick sections were cut with a Polycut E microtome (Reichert-Jung, Heidelberg, Germany). The sections were deplastified with ethylene glycol monoethyl acetate to allow for optimal staining. In each sample, two consecutive sections were selected that were stained with Masson Goldner Trichrome for static parameters or mounted unstained for the measurement of dynamic parameters using fluorescence microscopy.

Histomorphometric measurements in mice were carried out using a digitizing table with Osteomeasure® software (Osteometrics Inc., Atlanta, GA, USA). In addition to standard histomorphometric parameters, we measured cartilage volume per bone volume. This represents the relative amount of growth plate material that persists within secondary trabeculae. Nomenclature and abbreviations follow the recommendations of the American Society for Bone and Mineral Research [13].

Microcomputed tomography

Right femurs were scanned in phosphate buffered saline using cone beam CT (Skyscan 1172) at a voxel size of 6 μm. Scan parameters included a 0.45-degree increment angle, 3 frames averaged, an 84-kVp and 118-mA X-ray source with a 0.5-mm Al filter to reduce beam hardening artifacts. Trabecular bone was analyzed in a region starting at 0.5 mm proximal of the distal femoral growth plate (to avoid primary spongiosa) and scanning a 1 mm section of bone in a proximal direction. Trabecular bone was manually selected along the inner cortical surface. Scans were quantified using the system's analysis software (Skyscan CT Analyser, Version 1.11.8.0).

To analyze cortical bone, scanning was performed starting at a distance of 44% of the total femur length from the distal end and scanned for 1 mm proximally. Average outer bone diameter and average diameter of the bone marrow cavity were determined from cross-sectional areas assuming a circular bone cross-section. Cortical thickness was calculated as the difference of these two diameters divided by 2.

Biomechanical testing

Following microCT scanning, right femora were loaded to failure in three-point bending using a Mach-1™ micromechanical testing system (Biomomentum Inc., Laval, Canada). The specimens were thawed one day prior to the test and all the muscle tissue cleaned off. The bone was soaked overnight in phosphate buffered saline at room temperature until mechanical testing. The mid-diaphysis was loaded under tension. The distance between the lower supports was 7 mm with a vertical displacement rate of 50 μm/s.

Quantitative backscattered electron imaging (qBEI) analyses

The qBEI analyses were performed at the left femur. Bone mineralization density distribution (BMDD) was determined in the metaphyseal spongiosa, the epiphyseal spongiosa and in the midshaft cortical bone, as described before [14,15]. Bone areas were imaged using a digital electron microscope (DSM 962, Zeiss, Oberkochen, Germany) and gray level histograms were deduced, which were transformed into calcium weight percent (wt.% Ca) histograms. The derived BMDD parameters were the mean calcium concentration (CaMean; weighted mean), the most frequently occurring calcium concentration (CaPeak; the peak position of the BMDD) in the sample, the width of the BMDD distribution (CaWidth; full width at half maximum) reflecting the heterogeneity in matrix mineralization, the portion of low mineralized bone (CaLow; the percentage of bone area mineralized below 17.68 wt.% Ca,