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.

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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 bisphosphate 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 (Brlt mouse, with a G349C mutation in Col1a1 and a mouse harboring a G609C mutation in Col1a2) [7–9].
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 for Children in 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 microcomputer 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 epiphysial 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,
corresponding to bone matrix of primary mineralization) and the portion of high mineralized bone (Calligh; the percentage of bone mineralized above the 95th percentile of the WT control BMDD).

**Statistics**

For serum biochemistry, bone histomorphometry, microCT and mechanical testing, differences between the four groups of animals at each of the two ages were assessed for significance using ANOVA. The least significant difference method was used for post-hoc comparisons between two groups. qBEI data were evaluated with 2-way ANOVA and the Bonferroni post-hoc test. For all analyses, \( P < 0.05 \) was considered significant.

**Results**

Pretreatment body weight was significantly lower in OI mice compared to WT mice (Table 1). Sost-ab had no effect on weight gain during the study interval (\( P > 0.10 \)) or on weight or femur length at the end of the study. In untreated mice, serum sclerostin concentrations were similar between OI and WT mice at 8 weeks of age and significantly higher levels at 24 weeks (Fig. 1A). No treatment-associated differences in PINP levels were observed in OI mice. Serum CTX levels were not influenced by Sost-ab in either WT or OI mice. Serum activity of alkaline phosphatase was higher in Sost-ab treated WT than in untreated WT mice at 8 weeks (\( P = 0.04 \)) but not at 24 weeks of age. No treatment effects with regard to alkaline phosphatase activity were noted for OI mice (data not shown). Serum calcium and phosphorus concentrations were similar between all groups (data not shown).

**Serum biochemistry**

Serum markers of bone metabolism, PINP and CTX, were significantly higher in untreated OI mice than in untreated WT mice (Fig. 1A). Relative to untreated WT mice, WT mice receiving Sost-ab had marginally higher PINP concentrations at 8 weeks of age and significantly higher levels at 24 weeks (Fig. 1A). For serum biochemistry, bone histomorphometry, microCT and mechanical testing, differences between the four groups of animals at each of the two ages were assessed for significance using ANOVA. The least significant difference method was used for post-hoc comparisons between two groups. qBEI data were evaluated with 2-way ANOVA and the Bonferroni post-hoc test. For all analyses, \( P < 0.05 \) was considered significant.

**Lumbar vertebra analyses**

L4 histomorphometry showed a positive effect of Sost-ab on trabecular bone volume in younger WT mice, but not in OI mice of the same age or in older mice of either genotype (Fig. 1B). All dynamic bone formation parameters (mineral apposition rate, mineralizing surface, bone formation rate) were lower in untreated OI mice than in untreated WT mice in the younger age group (Fig. 1B). Sost-ab had no detectable effect on these parameters in L4 trabecular bone.

**Femur analyses**

At the distal femur, Sost-ab treatment was associated with higher trabecular bone volume in WT mice at both ages and in 8-week-old OI mice, but not in 24-week-old OI mice (Fig. 2A). Dynamic bone formation parameters were lower in untreated OI mice relative to untreated WT mice (Fig. 2A). Sost-ab treatment was associated with higher bone formation rate in WT mice but not in OI mice. Untreated OI mice had a larger cartilage volume per bone volume than untreated WT mice at 8 weeks of age (12.6% [2.4] vs. 7.6% [0.8]; \( P = 0.02 \)), but not at 24 weeks. Cartilage volume per bone volume was not influenced by Sost-ab treatment (data not shown).

At the midshaft femur, Sost-ab treatment was associated with higher cortical thickness in WT mice at both ages, which was due to numerically larger outer bone diameter and smaller size of the marrow cavity (Table 1). In OI mice, the Sost-ab treated group had higher cortical thickness at 8 weeks, due to a non-significantly smaller marrow cavity.

**Mechanical testing**

Three-point bending tests of the right femur showed that in WT but not in OI mice, Sost-ab was associated with higher ultimate load and work to failure (Fig. 2B). In 4 of the 8 OI mice that started Sost-ab at 20 weeks of age, the femur was fractured in vivo or during the removal of muscle tissues in preparation for the three-point bending test. Therefore, mechanical testing was not performed in these 4 OI mice. No evidence of femur fracture was detected in the 8 OI mice receiving control injections.

**Quantitative backscattered electron imaging analyses at the femur**

At all skeletal sites investigated (trabecular bone at the metaphysis, trabecular bone at the epiphysis, cortical bone at the diaphysis) BMDs showed a clear shift towards higher mineralization in OI mice compared to WT mice (Fig. 3). At both 8 and 24 weeks of age, CaPeak was higher in OI than in WT mice at all three measurement sites (between 2.8% and 8.3%) (Figs. 4 and 5). CaWidth, which reflects the heterogeneity of mineralization, was about 30% lower in OI mice compared to WT mice in the cortical region at both ages where also an increase of CaHigh was observed (+69% to +78%). In both genotypes there was a general increase in bone matrix mineralization from the age of 8 weeks to 24 weeks.

Regarding the effect of Sost-ab on BMD parameters, no effect on CaPeak was found, regardless of genotype, age or measurement location. However, in 8-week-old mice, Sost-ab treatment was associated with higher CaMean in metaphyseal bone of WT mice and in epiphysial bone of OI mice (Fig. 4). This was accompanied by a decrease in Calow in these regions. None of the BMD parameters varied with Sost-ab treatment in 24-week old mice (Fig. 5).

**Discussion**

In this study we observed that mice with severe dominant OI caused by a splice site mutation in Col1a1 had a smaller response to Sost-ab therapy than their WT littermates. In OI mice, Sost-ab did not have a detectable effect on serum markers of bone metabolism. The treatment
Fig. 1. Serum biochemistry (A) and L4 vertebral histomorphometry (B). N = 8 mice per group. * p < 0.05 Sost-ab versus control in WT; + p < 0.05 OI control versus WT control.

Fig. 2. Femur analyses. (A) Results of microCT (BV/TV) and dynamic histomorphometry MAR, MS/BS, BFR/BS) in trabecular bone at the distal femur. (B) Results of three-point bending tests. N = 8 mice per group. * p < 0.05 Sost-ab versus control in WT; + p < 0.05 OI control versus WT control; # p < 0.05 Sost-ab versus control in OI.
Fig. 3. Backscattered electron imaging at the distal femur (longitudinal section) of 8 week-old mice: Untreated wild type (upper panel) showing the three regions of interest in which BMDD analysis was performed: MS, cancellous metaphyseal bone; ES, cancellous epiphyseal bone; Ct, cortical midshaft bone. Metaphyseal cancellous bone of untreated (lower left panel) vs. treated (lower right panel) wild type individuals. Examples of BMDD histograms: Analysis at the three skeletal sites for the four experimental groups.

Fig. 4. Results of qBEI analyses at the distal femur in 8 week-old mice of the metaphyseal spongiosa (MS), epiphyseal spongiosa (ES) and cortical bone (Ct). N = 8 mice per group. *** p < 0.0001, ** p < 0.005, * p < 0.05 vs. genotype and same treatment; °°° p < 0.0001, °° p < 0.005, ° p < 0.05 vs. treatment and same genotype using Bonferroni post-hoc tests.
also had little effect on bone formation activity as determined by
dynamic histomorphometry and on bone matrix mineralization as
measured by qBEI. We did however observe some effect of Sost-ab on
femoral cortical thickness and trabecular bone volume in OI mice
that were treated during growth. In contrast, Sost-ab in WT mice had
a robust effect on bone formation, bone mass and bone strength.

Serum PINP, a bone formation marker, was higher in untreated OI
mice than in untreated WT mice, but dynamic histomorphometry at
both the femur and the lumbar spine indicated that OI mice had low
bone formation activity. PINP mirrors the production of procollagen
type I [16], while histomorphometric bone formation rate depends on
the rate of extracellular matrix deposition on trabecular bone surfaces.
The discrepancy between these two indicators therefore suggests that
procollagen type I is produced and secreted at a high rate (leading
to high circulating PINP) but that a large proportion of this procollagen
is not integrated into the extracellular bone matrix (leading to
low bone formation rate on histomorphometry). It therefore appears
that a substantial proportion of the procollagen type I secreted by
Col1a1Jrt/+ osteoblasts does not contribute to bone formation. Our
results thus indicate that Sost-ab treatment of OI mice did not have a
detectable effect on systemic procollagen secretion or on local bone for-
mation rates in trabecular bone at the distal femur or at L4 vertebral
bodies.

Despite their manifested defect in bone formation, OI mice had ele-
vated serum levels of the bone resorption marker CTX [17]. This is in line
with human and murine studies that have demonstrated elevated bone
resorption in OI [18,19]. CTX serum levels did not change with Sost-ab
treatment, indicating that the intervention had no effect on bone
resorption activity. Accordingly, Sost-ab did not have an effect on the
amount of cartilage in the metaphyseal spongiosa of the distal femur
of growing mice. Cartilage at that site is a remnant of growth plate
cartilage and accumulates when antiresorptive treatment, such as
biphosphonates, is administered during growth [20]. This potential
adverse effect on bone quality was thus not observed with Sost-ab.

We found that Col1a1Jrt/+ mice had an increased bone matrix min-
eralization compared to WT mice. Matrix hypermineralization is a con-
sistent feature of bone tissue affected by direct or indirect abnormalities
in collagen type I, both in humans [15,21–23] and in mice [24,25] and
might contribute to bone brittleness. Sost-ab treatment seemed to
have a minor effect on BMDD, which was limited to trabecular bone
in young mice. This likely refers to an increase in bone volume which
results in a higher average tissue age. Regarding the 24-week-old mice, no
treatment effects were observed at any sites.

Despite ineffective bone formation, Sost-ab treatment seemed to
confer some benefit in younger OI mice, as their trabecular bone volume
and cortical thickness were higher than in untreated OI mice. These
effects were not seen in older OI mice, which could be related to the obser-
vation that older OI mice had low serum sclerostin levels, thus
decreasing the scope of change that sclerostin inhibition could achieve.
In contrast, younger OI mice had similar serum sclerostin concentrations
as WT mice. It is interesting to note that these variations in serum
sclerostin levels in OI mice mirror recent reports in humans with OI,
where normal sclerostin serum concentrations were found in children
and adolescents but low values in adults [26,27]. The mechanisms
explaining these observations are not clear at present, but they could in-
dicate that sclerostin inhibition might have more effect during growth
than in adults with OI.

Nevertheless, even in younger OI mice, the additional cortical bone
observed after Sost-ab treatment seemed to be located on the
endocortical surface where it has less effect on resistance to bending.
This may explain why biomechanical testing did not reveal a positive
treatment effect in OI mice. Also, Sost-ab had no effect on matrix mineral-
alization of femoral cortical bone in the OI mouse and thus presumably
did not alter the underlying bone brittleness.
The effects of Sost-ab treatment in our Col1a1Jrt/+ mouse model are less than what had been reported by Sinder et al. and Jacobsen et al., who used different mouse models of OI [7,8]. One explanation for the difference between studies may be that these mice had less severe bone fragility than the Col1a1Jrt/+ mouse. For example, our mechanical testing showed that ultimate load was 64% lower in 8 week old untreated Col1a1Jrt/+ mice than in untreated WT mice, whereas ultimate load was decreased by only 33% in the two other OI mouse models [7,8]. It is possible that the more severe collagen abnormality in the Col1a1Jrt/+ mouse prevented osteoblasts from responding to Sost-ab. Other differences between studies include the antibody used and the route and duration of its administration (intravenous for 4 weeks in the present study, subcutaneous for 2 and 6 weeks, respectively, in the other two studies) and the age of the mice (8 or 24 weeks at the study endpoint in the present study, 10 weeks in the study by Sinder et al., 12 weeks in the study by Jacobsen et al.). Nevertheless, the results of Sost-ab in our WT mice were similar to the WT mice reported by the other two studies and the age of the mice (8 or 24 weeks at the study endpoint in the present study, subcutaneous for 2 and 6 weeks, respectively, in the other two studies) and the age of the mice (8 or 24 weeks at the study endpoint in the present study, 10 weeks in the study by Sinder et al., 12 weeks in the study by Jacobsen et al.). Nevertheless, the results of Sost-ab in our WT mice were similar to the WT mice reported by Sinder et al. and Jacobsen et al., suggesting that the main explanation for differential study effects lies in the disease severity of the OI model used rather than in the treatment approach. In analogy, a recent clinical study showed that bone anabolic therapy with parathyroid hormone had a better effect in milder than in severe forms of OI [28].

In summary, we found that Sost-ab treatment had a larger effect in WT mice than in Col1a1Jrt/+ mice. Sost-ab did not correct the bone formation deficits in OI mice nor did it have a major effect on bone mass, even though marked improvements had previously been reported in OI mouse models with a milder phenotype. Our data therefore suggest that Sost-ab is less effective in the more severely affected Col1a1Jrt/+ mouse model of OI.

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