Bone 101 (2017) 96–103

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

Combination sclerostin antibody and zoledronic acid treatment outperforms either treatment alone in a mouse model of osteogenesis imperfecta

David G. Little [a,b,*], Lauren Peacock [a], Kathy Mikulec [a], Michaela Kneissel [c], Ina Kramer [c], Tegan L. Cheng [a,b], Aaron Schindeler [a,b], Craig Munns [a,b]

[a] Orthopaedic Research & Biotechnology Unit, The Children's Hospital at Westmead, Sydney, NSW, Australia
[b] Discipline of Paediatrics and Child Health, Faculty of Medicine, University of Sydney, Sydney, NSW, Australia
[c] Bone Unit, Musculoskeletal Disease Area, Novartis Institutes for BioMedical Research, Basel, Switzerland

ARTICLE INFO

Article history:
Received 30 January 2017
Revised 26 April 2017
Accepted 27 April 2017
Available online 29 April 2017

Keywords:
Osteogenesis imperfecta
Zoledronic acid
Sclerostin
Scl-Ab antibody
Col1a2 G610C

ABSTRACT

In this study, we examined the therapeutic potential of anti-Sclerostin Antibody (Scl-Ab) and bisphosphonate treatments for the bone fragility disorder Osteogenesis Imperfecta (OI). Mice with the Amish OI mutation (*Col1a2 G610C* mice) and control wild type littermates (WT) were treated from week 5 to week 9 of life with (1) saline (control), (2) zoledronic acid given 0.025 mg/kg s.c. weekly (ZA), (3) Scl-Ab given 50 mg/kg IV weekly (Scl-Ab), or (4) a combination of both (Scl-Ab/ZA). Functional outcomes were prioritized and included bone mineral density (BMD), bone microarchitecture, long bone bending strength, and vertebral compression strength. By dual-energy absorptiometry, Scl-Ab treatment alone had no effect on tibial BMD, while ZA and Scl-Ab/ZA significantly enhanced BMD by week 4 (+16% and +27% respectively, $P < 0.05$). Scl-Ab/ZA treatment also led to increases in cortical thickness and tissue mineral density, and restored the tibial 4-point bending strength to that of control WT mice. In the spine, all treatments increased compression strength over controls, but only the combined group reached the strength of WT controls. Scl-Ab showed greater anabolic effects in the trabecular bone than in cortical bone. In summary, the Scl-Ab/ZA intervention was superior to either treatment alone in this OI mouse model, however further studies are required to establish its efficacy in other preclinical and clinical scenarios.

1. Introduction

Osteogenesis Imperfecta (OI) is a genetic disorder that features bone fragility and decreased bone mass. The severity of this condition can vary widely, from very mild forms with infrequent fractures to highly deforming and, on occasion, fatal phenotypes [1]. While bone is the primary tissue affected, extraskeletal tissues such as the sclera of the eye, teeth, skin and ligaments can all exhibit abnormalities. OI is conventionally associated with a reduction in collagen or dysfunctional collagen mutants. Although the majority of cases are attributable to mutations in the *COL1A1* or *COL1A2* genes, a range of other genetic causes for OI have emerged [2]. Many of these alternative gene mutations are associated with distinct clinical patterns of severity, progression, and extraskeletal tissues affected.

The clinical management of children with OI is dependent on the severity of their disease. It typically aims to reduce fracture rates by minimizing trauma and risk of impact injuries while concurrently attempting to maximize their bone strength [3]. Bisphosphonate intervention is a highly studied approach that can achieve significant increases in bone mass in the context of OI [4]. However, a large multicenter randomized placebo-controlled study in moderate-to-severe OI that examined the efficacy of oral alendronate failed to demonstrate decreases in fracture rates, despite significant improvements in bone mineral density [5]. Similarly, a meta-analysis of multiple bisphosphonate trials for OI found that the benefits of treatment for fracture rate endpoints were inconclusive [6]. Thus, there is clear basis for the investigation of alternate agents and therapies for OI.

Sclerostin is a protein that is a key negative regulator of bone growth. Mutations in *SOST*, the gene encoding Sclerostin, lead to diseases of high bone mass [7]. Several groups have developed anti-Sclerostin monoclonal antibodies (Scl-Ab) for the management of osteoporosis, and to date trials have shown potent effects to increase bone mass and reduce fracture risk [8]. Due to its potent ability to increase bone growth, it has been proposed that monoclonal Scl-Ab may have utility for managing other metabolic bone diseases [9,10] and improving bone repair [11].

* Corresponding author at: Orthopaedic Research & Biotechnology, Research Building The Children's Hospital at Westmead, Locked Bag 4001, Westmead, NSW 2145, Australia.
E-mail address: david.little@health.nsw.gov.au (D.G. Little).

http://dx.doi.org/10.1016/j.bone.2017.04.016
8756-3282/

To investigate the impact of Scl-Ab in the context of OI, we performed a controlled study in a relevant mouse model. A recent model of mild-to-moderate severity, dominant negative OI, using the "Amish variant" mouse with a G610C mutation in the *Col1a2* gene was selected [12]. Compared to other models, such as the *oim* mouse [13], this strain has a milder phenotype but still exhibits significant decreases in bone mineral density and mechanical strength [12]. In a paper published by Jacobsen et al. in 2014, indicated that mature mice that received 6 weeks of Scl-Ab showed statistically significant increases in trabecular BV/TV, cortical thickness, and mechanical properties [14].

In this study we sought to extend upon the work of Jacobsen et al. via a preclinical model that may better represent the common pediatric OI situation. Skeletally immature, 5 week old mice were treated with Scl-Ab alone and/or with a bisphosphonate. Bisphosphonate modulation of bone resorption remains a common approach to maximizing bone strength in pediatric OI. Many patients eligible for Scl-Ab treatment may already have received or still receiving these anti-resorptive agents. We hypothesized that the combination of Scl-Ab and the potent bisphosphonate Zoledronic Acid (ZA) may lead to improved outcomes over either treatment alone.

2. Methods

2.1. Col1a2G610C mouse colony

The *Col1a2G610C* strain was supplied by Prof J Bateman (Murdoch Institute, VIC, Australia) and was sourced from the originally published line [12]. The mouse was maintained on a C57BL6/J background. The *Col1a2G610C* mutation is dominant negative and breeding pairs favored male studs carrying the *Col1a2G610C* mutation. Mice were bred in house in the CHW Transgenic Facility and were given standard mouse chow and water ad libitum. Genotyping was performed on ear punch tissue by TransnetYX, Inc. (TN, United States) using The Jackson Laboratory primer sets.

2.2. Pharmaceuticals

ZA and Scl-Ab monoclonal antibody were supplied by Novartis Pharma AG (Basel, Switzerland). This Scl-Ab has proven efficacy in murine models [15].

2.3. Study design

Female *Col1a2G610C* (OI) mice and their wild type littermates (WT) were treated from week 5 to week 9 of life with either saline (control), ZA, Scl-Ab, or a combination of both agents (Scl-Ab/ZA). Group sizes were $n = 10$ mice. ZA was given 0.025 mg/kg s.c. weekly and Scl-Ab given 50 mg/kg IV weekly. Animals were monitored by weekly X-ray (Faxitron MX5, Faxitron Corp., IL, USA) for spontaneous fractures and one mouse was excluded due to spontaneous fracture during the time course of the study. Mice were dosed with calcein (10 mg/kg) at 10 and 3 days prior to the study end. At the conclusion of the study, animals were euthanized by CO_2 asphyxiation and tissues collected for analysis. Specimens for mechanical endpoints were stored frozen in saline soaked gauze and other specimens were fixed in 4% paraformaldehyde (PFA) and stored in 70% ethanol.

2.4. Radiographic outcomes

Dual-energy x-ray absorptiometry (DEXA) was performed weekly on live animals to measure areal Bone Mineral Density (BMD) (GE Lunar PIXImus, Little Chalfont, UK). Analyses were performed using the proprietary Lunar PIXImus software on the tibiae and spine.

MicroCT analysis was performed on harvested specimens in saline using a SkyScan 1174 benchtop scanner (Bruker, Kontich, Belgium). Standard conditions for microCT analysis for murine bones were used as previously published [16]. Tibiae were scanned at an isotropic voxel resolution of 12 μm with a 0.5 mm aluminium filter, 50 kV x-ray tube voltage, 800uA tube electric current and 4500 ms exposure time. A global threshold of 0.3 g/mm3 was set for bone tissue 3D volumetric analysis and modeling were carried out using CTAn Software version 1.11.8.0 (Bruker). For the cortical region we measured 5 mm below the growth plate and continuing distally another 0.5 mm. For the trabecular bone, we measured 0.5 below the growth plate and continuing distally another 1.2 mm.

2.5. Histology and histomorphometry

The tibia were cryo-sectioned onto cryofilm and measured on the Bioquant Image Analysis software (R&M Biometrics, Nashville, TN, USA) at 10×. We measured in the trabecular region of the tibia 0.5 mm down from the growth plate for BFR, MAR and Mineralized Surface. Images were captured using an Aperio Fluorescent Slide Scanner (Leica Biosystems, Wetzlar, Germany).

2.6. Mechanical testing

Tibiae and vertebrae were harvested and stored in saline soaked gauze at −80 °C allowed to thaw to room temperature prior to testing. Mechanical testing protocols were based on previously published methods [17]. Testing was performed using an Instron 5944 (Instron Corp., Norwood, MA, USA), with data collected using BlueHill 3 software.

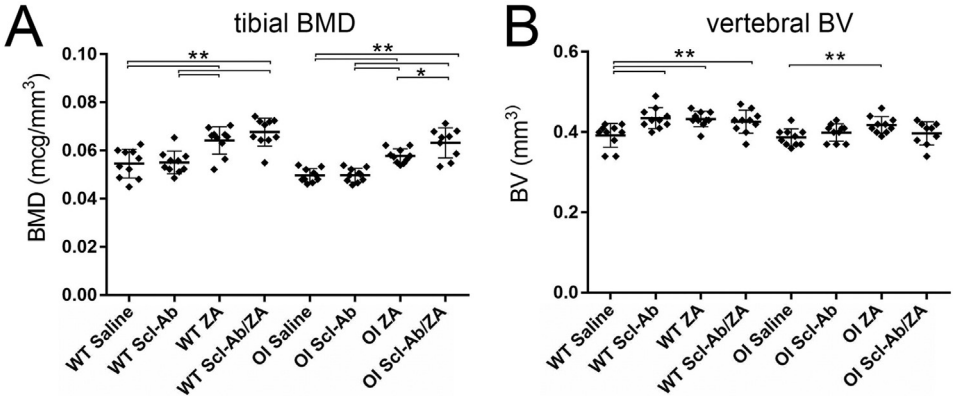

Fig. 1. Bone mineral density (BMD) of the tibiae (A) and bone volume (BV) of the spine (B) after 4 weeks of treatment starting week 5 in WT and OI mice. $^{*}P < 0.05$, $^{**}P < 0.01$.