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Sclerostin antibody inhibits skeletal deterioration in mice exposed to partial weight-bearing

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ABSTRACT

Whereas much is known regarding the musculoskeletal responses to full unloading, little is known about the physiological effects and response to pharmacological agents in partial unloading (e.g. Moon and Mars) environments. To address this, we used a previously developed ground-based model of partial weight-bearing (PWB) that allows chronic exposure to reduced weight-bearing in mice to determine the effects of murine sclerostin antibody (SclAbII) on bone microstructure and strength across different levels of mechanical unloading. We hypothesize that treatment with SclAbII would improve bone mass, microarchitecture and strength in all loading conditions, but that there would be a greater skeletal response in the normally loaded mice than in partially unloaded mice suggesting the importance of combined countermeasures for exploration-class long duration spaceflight missions. Eleven-week-old female mice were assigned to one of four loading groups: normal weight-bearing controls (CON) or weight-bearing at 20% (PWB20), 40% (PWB40) or 70% (PWB70) of normal. Mice in each group received either SclAbII (25 mg/kg) or vehicle (VEH) via twice weekly subcutaneous injection for 3 weeks. In partially-unloaded VEH-treated groups, leg BMD decreased −5 to −10% in a load-dependent manner. SclAbII treatment completely inhibited bone deterioration due to PWB, with bone properties in SclAbII-treated groups being equal to or greater than those of CON, VEH-treated mice. SclAbII treatment increased leg BMD from +14 to +18% in the PWB groups and 30 ± 3% in CON ($p < 0.0001$ for all). Trabecular bone volume, assessed by μCT at the distal femur, was lower in all partially unloaded VEH-treated groups vs. CON-VEH ($p < 0.05$), and was 2–3 fold higher in SclAbII-treated groups ($p < 0.001$). Midshaft femoral strength was also significantly higher in SclAbII vs. VEH-groups in all-loading conditions. These results suggest that greater weight bearing leads to greater benefits of SclAbII on bone mass, particularly in the trabecular compartment. Altogether, these results demonstrate the efficacy of sclerostin antibody therapy in preventing astronaut bone loss during terrestrial solar system exploration.

1. Introduction

The profound effects of mechanical unloading on muscle atrophy and skeletal fragility are well established (Wolff, 1892). However, there has been little investigation into the physiological effects of partial reduction of weight-bearing (Ellman et al., 2013; Wagner et al., 2010; Swift et al., 2013) that will be encountered during long duration exploration class missions to the Moon or Mars. Minimizing the risk of fracture and maximizing the ability of crewmembers to perform critical tasks safely throughout the mission and preventing premature osteoporosis upon return to Earth is critically important. The optimization of mission designs for long duration human spaceflight requires determining the best combination of existing and proposed spacecraft engineering countermeasures (e.g. exercise equipment, artificial gravity) with pharmacologic countermeasures that could offer optimal

Abbreviations: Tb.BV/TV, bone volume fraction; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; ConnD, connectivity density; SMI, structure model index; Tt.Ar, total cross-sectional area; Ct.Ar, cortical bone area; Me.Ar, medullary area; Ct.Ar/Tt.Ar, cortical bone area fraction; Ct.Th, cortical thickness; pMOI, polar moment of inertia; TMD, tissue mineral density.

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efficacy while minimizing mass, volume, and energy, and maintaining crew-member task scheduling flexibility. To address the gap in knowledge effects of partial gravity on the musculoskeletal system and the unknown risks it presents to astronauts in terrestrial space environments, our group previously developed the partial weight-bearing (PWB) model (Ellman et al., 2013; Wagner et al., 2010; Macias et al., 2016) that enables long-term exposure of mice to partial loading while maintaining quadrupedal locomotion. We, and others (Swift et al., 2013), have used this model to show that bone and muscle loss is linearly proportional to the reduction of mechanical loads (Ellman et al., 2013).

The osteocyte-secreted protein sclerostin inhibits bone formation (van Bezooijen et al., 2005; Collette et al., 2012; Galli et al., 2010; Gardner et al., 2005). Humans with genetic mutations leading to sclerostin deficiency have increased bone mass (Gardner et al., 2005; Balemans et al., 2005; Balemans et al., 2001; Balemans et al., 2002; Brunkow et al., 2001; Hamersma et al., 2003; Staehling-Hampton et al., 2002), and in rodents, inhibition of sclerostin via pharmacologic antibody treatment (Ominsky et al., 2010; Li et al., 2010; Li et al., 2009; Spatz et al., 2013; Tian et al., 2011) or genetic manipulation (Lin et al., 2009) leads to increased bone formation. Moreover, treatment with sclerostin antibody increases bone mass and reduces fracture risk in postmenopausal osteoporotic women (Cosman et al., 2016). In addition, sclerostin levels are responsive to mechanical loading, with increased expression with mechanical unloading and decreased expression with increased loading (Spatz et al., 2013; Robling et al., 2006; Smith et al., 2012). We previously reported that in mice subjected to mechanical unloading via hindlimb unloading (HLU), treatment with sclerostin antibody not only inhibits bone loss, but leads to improved bone mass via increased bone formation (Spatz et al., 2013). For a few bone outcomes, we observed greater anabolic effects of sclerostin inhibition in the normally loaded mice (Spatz et al., 2013). Notably, sclerostin protein expression is increased in cortical bone in mouse models of unloading-induced bone loss (Swift et al., 2013; Macias et al., 2016) and SOST downregulation is required for the osteogenic response for mechanical loading (Tu et al., 2012). To further investigate the skeletal responses to sclerostin inhibition in a mechanical unloading environment that simulates terrestrial space exploration, we tested the ability of sclerostin antibody to inhibit skeletal deterioration during exposure to partial weight-bearing at 20% (approximate Moon simulation), 40% (approximate Mars simulation), and 70% of normal loading. We hypothesize that treatment with sclerostin antibody would improve bone mass, microarchitecture and strength in all loading conditions, but that there would be a greater skeletal response in the normally loaded mice than in partially unloaded mice suggesting the importance of combined countermeasures for exploration-class long duration spaceflight missions.

2. Materials and methods

2.1. Overview of study design

We tested the ability of sclerostin antibody (SclAbII) to prevent bone loss in female mice (C57Bl/6J, 11 weeks of age (Jilka, 2013)) subjected to mechanical unloading for 21 days. Female mice were chosen as they appear to tolerate the partial weight-bearing model better than males (Ellman et al., 2013; Wagner et al., 2010). Mice were assigned to one of four loading groups ($n = 6$ to 17/group): partial weight-bearing at 20% (PWB20), 40% (PWB40), 70% (PWB70) of normal weight-bearing, or control (CON, normal weight-bearing). Animals were assigned to groups by total body bone mineral density (BMD) and body mass in a manner to minimize differences between groups at baseline. Mice in each group were subcutaneously injected with either SclAbII at 25 mg/kg (Ominsky et al., 2010; Li et al., 2010; Li et al., 2009) or vehicle (VEH) twice weekly. In brief, the first injection was given the day of unloading (Tuesday), the volume injected for a median 20 g mouse used in the study was 18.2 ul of a 27.4 mg/ml SclAbII stock solution or vehicle, and volume of the dose was weight adjusted based on the prior day's animals weight. Prior to unloading the animals' weight was recorded on the first day of unloading, inclusion of the partial weight bearing harness weight, to allow for accurate weight and dose adjustments throughout the experiment. Subsequent dosing occurred on biweekly injection schedules occurring every Tuesday and Friday during the 21-day experiment. All mice had access to standard rodent chow and water ad libitum. The protocol was approved by the Institutional Animal Care and Use Committee at Beth Israel Deaconess Medical Center.

2.2. Partial weight-bearing (PWB) model

For partial weight-bearing, we followed the methods described previously by Ellman et al. (2013), Wagner et al. (2010) and Swift et al. (2013). In brief, two to three days prior to unloading, mice assigned to PWB groups were placed in the PWB jacket and singly housed in standard vivarium cages for acclimation. On day 0, mice were placed in a two-point full suspension rig, as described previously (Ellman et al., 2013; Wagner et al., 2010; Swift et al., 2013). A clasp on the jacket and a tail wrap were connected by a chain and spaced by a hollow metal rod to distribute loading. This apparatus was then joined to a spring and hung from a wheel with linear freedom along a rail across the top of a cage. Adjustments to actual weight-bearing, or effective mass, were made by threading the spring through its support thereby changing the length of spring engaged by the harness, and providing differential vertical force to support mouse's body weight. Minor adjustments, less than 5% of desired effective mass drift daily, were measured and corrected by quiet standing on a scale at the desired partial weight and the spring tension adjusted to maintain the desired amount of unloading. Animals (1 PWB20-VEH, 3 PWB70-VEH, 2 PWB70-SclAbII) who were non-compliant recorded as greater than a 5% effective mass drift recorded more than once or with an escape from the partial weight-bearing harness more than once were excluded from all analyses. Prior work has verified the ground reaction forces and gait kinetics for the partial weight bearing model utilizing these methods (Ellman, 2014).

2.3. Bone mineral density and muscle mass

In vivo assessment of total body (exclusive of the head region) and leg (femur and tibia exclusive of femoral neck and foot) bone mineral density (BMD, g/cm^2) was performed at baseline and end of the study using peripheral dual-energy X-ray absorptiometry (pDXA, PIXImusII, GE Lunar Corp.), as previously described (Ellman et al., 2013; Spatz et al., 2013). Muscle atrophy was assessed by wet weight of the soleus and gastronemious muscles at necropy. Muscle mass was normalized to animal body weight.

2.4. Specimen harvesting / preparation

At the end of the study, mice were euthanized via CO_2 overdose. Femurs were harvested and cleaned of soft tissue. The right femur was prepared for imaging and biomechanical testing by wrapping in saline-soaked gauze and freezing at −20 °C.

2.5. Serum markers of bone metabolism

Mice were fasted for 2 h before blood was collected at the time of euthanasia and used to measure serum sclerostin (in vehicle