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Sclerostin antibody prevented progressive bone loss in combined ovariectomized and concurrent functional disuse

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ABSTRACT

Osteoporosis is characterized by low bone mass and compromised trabecular architecture, and is commonly occurred in post-menopausal women with estrogen deficiency. In addition, prolonged mechanical unloading, i.e., long term bed rest, can exaggerate the bone loss. Sclerostin is a Wnt signaling antagonist and acts as a negative regulator for bone formation. A sclerostin-neutralizing antibody (Scl-Ab) increased bone mineral density in women with postmenopausal osteoporosis and healthy men. The objective of this study was to characterize the condition of bone loss in ovariectomized (OVX) rats with concurrent mechanical unloading and evaluate the effect of sclerostin antibody treatment in mitigating the prospective severe bone loss conditions in this model. Four-month-old OVX- or sham-operated female SD rats were used in this study. They were subjected to functional disuse induced by hind-limb suspension (HLS) or free ambulance after 2 days of arrival. Subcutaneous injections with either vehicle or Scl-Ab at 25 mg/kg were made twice per week for 5 weeks from the time of HLS. μCT analyses demonstrated a significant decrease in distal metaphyseal trabecular architecture integrity with HLS, OVX and HLS + OVX (bone volume fraction decreased by 29%, 71% and 87% respectively). The significant improvements of various trabecular bone parameters (bone volume fraction increased by 111%, 229% and 297% respectively as compared with placebo group) with the administration of Scl-Ab are associated with stronger mechanical property and increased bone formation by histomorphometry. These results together indicate that Scl-Ab prevented the loss of trabecular bone mass and cortical bone strength in OVX rat model with concurrent mechanical unloading. The data suggested that monoclonal sclerostin-neutralizing antibody represents a promising therapeutic approach for severe osteoporosis induced by estrogen deficiency with concurrent mechanical unloading.

1. Introduction

Osteoporosis is characterized by compromised bone mass and microarchitectural deterioration [1–3]. Currently, it is estimated to affect more than 10 million Americans, cause about 1.5 million osteoporotic fractures each year and put an estimated 44 million Americans at risk, which exerts an extensive financial burden on the healthcare system with a direct annual medical cost of around 20 billion dollars [4]. With the growth of aging population, the incidence of osteoporosis and prevalence of osteoporotic fractures is expected to increase dramatically [5–8].

Significant bone loss related to mechanical disuse or loss of weight bearing in prolonged bed-ridden patients represents a critical issue [9–11]. Numerous biochemical parameters showed uncoupling between bone resorption and formation markers as well as an increased number of osteoclasts and enlarged resorption cavities in histomorphometric studies in prolonged immobilized subjects [10,12,13]. A considerable imbalance between bone formation and resorption induced by abrupt decline of ovarian production of estrogen in post-menopausal women represents another primary cause of the condition of osteoporosis [14]. Women over 50 years of age have also been reported as the most sedentary group of the adult populations [15]. Lack of mechanical stress and physical loading deteriorates bone integrity as exercise was proved to be beneficial to preserve bone mineral density (BMD) and prevent bone loss conditions [16,17]. The two risk factors above expose elderly female to greater risk of osteoporosis and fractures. Therefore, it is highly clinically relevant to examine the severe osteoporosis condition due to estrogen deficiency accompanied by concurrent mechanical unloading where more rapid and devastating bone mass deterioration is present, in conditions such as post-menopausal women who are confined to bed after the onset of stroke or accidents [10,18] and a better understanding of the interactions of

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the two risk factors may help lead to a better prevention and treatment of the disease.

Sclerostin, an osteocyte-expressed glycoprotein encoded by SOST gene, acts as a negative regulator of bone formation by antagonizing Wnt/β catenin signaling [2,19–22]. Both extended immobilization and condition of post-menopause has been related to an increase of sclerostin serum levels [10,22,23]. Patients with homozygous inactivation mutation of SOST gene develops sclerosteosis, a rare genetic disorder characterized by undetectable level of serum sclerostin, very high bone mass phenotype and nerve entrapment due to excessive bone formation. Individuals with heterozygous carriers for the mutation had increased bone mineral density and lower risk of fractures. This discovery has led to the hypothesis that sclerostin neutralizing agents might mimic the heterozygous carrier condition and be efficacious in reversing bone loss in osteoporosis [24–26]. Targeted deletion of SOST gene in mice resulted in increased bone formation, bone mass and bone strength whereas overexpression of SOST leads to an osteoporotic phenotype in mice [11,19,21,27].

It has been the emphasis of multiple recent studies to target Wnt/β catenin signaling pathway to promote bone formation [11,19,28]. Using monoclonal antibody to inhibit sclerostin seems to be a very promising strategy in that SOST is almost exclusively expressed in osteocytes [29,30]. Sclerostin-neutralizing monoclonal antibodies (Scl-Ab) have been tested in disuse-induced and OVX-induced osteoporosis animal models. In a right hind-limb-immobilization female rat model, histomorphometric data showed that Scl-Ab mediated blockade of sclerostin dramatically enhanced bone formation with an increased bone mass and reduced bone resorption [31]. In an aged OVX female rat model simulating postmenopausal osteoporosis, twice per week subcutaneous Scl-Ab injection at 25 mg/kg was administered for 5 weeks. Micro-CT and histomorphometry data exhibited significant increases in bone formation and bone mass comparing to the placebo groups. These changes together with improved stiffness and maximum load data obtained from destructive four-point bending tests indicate an improved skeletal strength [2].

All the evidences above consolidate the notion that Scl-Ab-mediated blockade of sclerostin represents a promising therapeutic approach for the treatment of osteoporosis induced by either immobilization or post-menopause. However, little is known about the effect of prolonged and severe osteopenia in bone loss induced by concurrent mechanical unloading and OVX. The efficacy of Scl-Ab in rats that suffered from severe osteoporosis with the presence of both prolonged immobilization and estrogen deficiency is yet to be determined. The aim of the study is to evaluate the effect of Scl-Ab in a severe progressive bone loss from combined estrogen deficiency and functional immobilization, such as under OVX with concurrent hindlimb suspension disuse in a rat model. Bone volume fraction, mechanical strength, bone formation rate, and plasma formation/resorption markers in such severe osteopenia and osteoporosis conditions were examined.

2. Materials and methods

All experimental procedures with the described animal models were approved by the Institutional Animal Care and Use Committee (IACUC) at Stony Brook University.

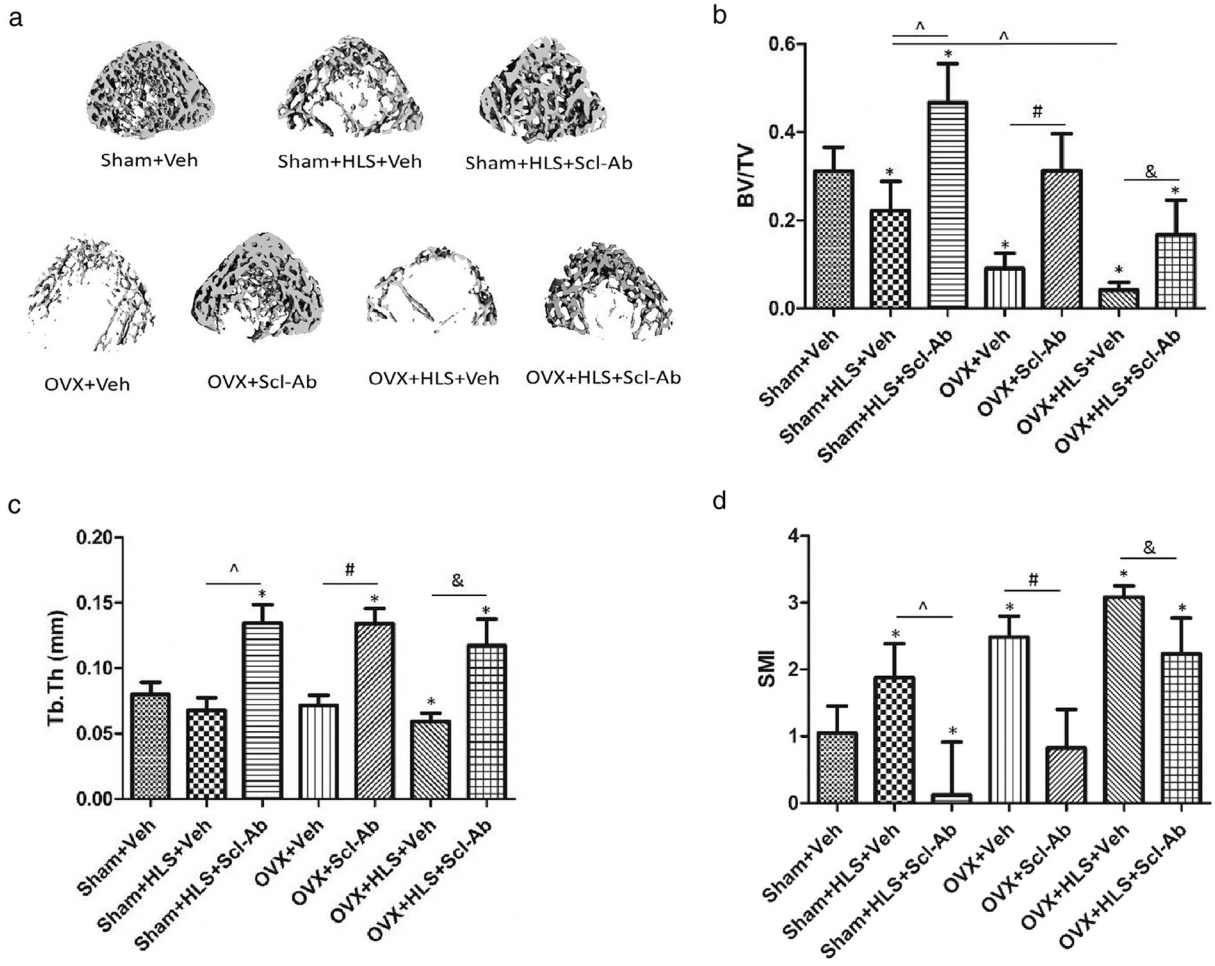

Fig. 1. a) μCT reconstruction images of trabecular area of distal femur of rats. b) Graphs show mean + SD values for bone volume fraction (BV/TV, %). c) Mean + SD values for trabecular thickness (Tb.Th, μm). d) Mean + SD values for SMI. $^{*}p < 0.05$ vs. Sham + Veh; $^{\wedge}p < 0.05$ vs. Sham + HLS + Veh; $^{\#}p < 0.05$ vs. OVX + Veh; $^{\&}p < 0.05$ vs. OVX + HLS + Veh.