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Sclerostin antibody treatment causes greater alveolar crest height and bone mass in an ovariectomized rat model of localized periodontitis

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ABSTRACT

Introduction: Periodontitis and osteoporosis are bone destructive diseases with a high prevalence in the adult population. The concomitant presence of osteoporosis may be a risk factor of progression of periodontal destruction. We studied the effect of sclerostin-neutralizing monoclonal antibody (Scl-Ab) on alveolar bone endpoints in an ovariectomized (OVX) rat model of induced experimental periodontitis.
Methods: Sixty female, 4-month-old Sprague–Dawley rats underwent sham operation or bilateral OVX and were left untreated for 2 months. Experimental periodontitis (ligature) was established by placing silk sutures subgingival to the right maxillary first and second molar teeth for 4 weeks, and feeding the rats food and high-sugar drinking water during this period. Thereafter, ligatures were removed and 25 mg/kg vehicle or Scl-Ab was administered subcutaneously twice weekly for 6 weeks. Rats were randomized into four groups: (1) Control (Sham + Vehicle), (2) Sham + Ligature + Vehicle, (3) OVX + Ligature + Vehicle, and (4) OVX + Ligature + Scl-Ab. Terminal blood and right maxilla specimens were collected for analyses.
Results: Group 3 rats showed lower bone volume fraction (BVF) of alveolar bone with higher bone resorption and lower bone formation than Group 2 rats. Group 4 rats had higher alveolar crest height, as assessed by linear distance of cementoenamel junction to the alveolar bone crest and greater alveolar bone mass using Micro CT, than Group 3 rats. Significantly higher values of mineral apposition rate (MAR) and mineralizing surface/bone surface (MS/BS) were also observed in Group 4 rats by analyzing polychrome sequential labeling data. Increased serum osteocalcin and osteoprotegerin, and deceased serum tartrate-resistant acid phosphatase and CTx-1 illustrate the ability of Scl-Ab to increase alveolar bone mass by enhancing bone formation and decreasing bone resorption in an animal model of estrogen deficiency osteopenia plus periodontitis.
Conclusion: Scl-Ab could be a potential bone anabolic agent for improving alveolar crest height and higher alveolar bone mass in conditions where alveolar bone loss in periodontitis is compounded by estrogen deficiency osteopenia.

1. Introduction

Periodontitis is characterized by loss of soft tissue attachment to teeth and resorption of alveolar bone [1]. The induced alveolar bone loss (ABL), considered as a hallmark of periodontitis, may cause loosening of tooth and finally lead to tooth loss, which in turn results in the progression of ABL and resorption of remaining residual ridge [2]. This chronic destructive disease is highly prevalent. According to the 2009–2010 National Health and Nutrition Examination Survey, approximately 47% of the adult population aged ≥30 years in the United States have periodontitis, with 30.0% and 8.5% in moderate and severe level, respectively [3]. Systemic bone loss may be a risk factor for decreased height of alveolar bone of periodontitis [4–6]. Since periodontitis and osteoporosis share multiple common risk factors and mechanisms [4,7], researchers have proposed that treatment of systemic bone destruction–related disease with bone anabolic agent could be a potential adjuvant therapy to reduce the rate of periodontal ABL [8,9].

Sclerostin, a protein secreted primarily by osteocytes, has been shown to negatively regulate osteoblast-mediated bone formation. One of its mechanisms involves competing with various Wnt ligands to prevent their binding to LRP5/6 coreceptors, thereby blocking intracellular activation of beta-catenin signaling [10,11]. Pharmacologic

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inhibition of sclerostin with a sclerostin monoclonal antibody (Scl-Ab) has been effective in increasing bone mass and strength in various animal models [12–16]. Clinical studies further suggest that administration of Scl-Ab to healthy men, postmenopausal women, or postmenopausal women with osteoporosis is well tolerated and leads to a dose-dependent increase in BMD at the hip and spine [17,18].

It has been reported that systemic Scl-Ab administration induced an anabolic response in alveolar bone under physiologic conditions and experimental periodontitis in rats [8]. These results for the first time proposed the therapeutic potential of sclerostin inhibition in facilitating bone regeneration in periodontal disease. Since almost one-third of US adults in the age group known to have osteoporosis also have moderate periodontitis [4], further clarification of the effect of Scl-Ab treatment on periodontitis-induced ABL and loss of alveolar bone mass associated with osteoporosis has great clinical implication. The present study aimed to investigate the anabolic effects of Scl-Ab in a rat model of ABL following induction of experimental localized periodontitis and estrogen deficiency osteopenia.

2. Materials and methods

2.1. Animals, treatment, and experimental design

Sixty female, 4-month-old, virgin Sprague–Dawley rats from the Department of Laboratory Animal Science at Shanghai Ninth People's Hospital were selected. The rats were received 2 weeks prior to the start of experiments and allowed to acclimatize in filter-top cages (three rats per cage), following a 12-hour light–dark cycle, with food (calcium 1.0%–1.8% and phosphorus 0.6%–1.2%; Shanghai Super — B&K Laboratory Animal Corp Ltd., Shanghai, China) and water available ad libitum. The Ethics Committee and the Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine approved the protocol and procedures performed.

Body weights were recorded before the initiation of the study and used for distribution so that each study group had similar mean body weight values (Fig. 1). There were fifteen rats in each group, five of which were used for tartrate-resistant acid phosphatase (TRAP) staining. The rats were either sham operated or bilaterally ovariectomized (OVX) thorough a vertical abdominal incision and left untreated for 2 months. Success of OVX was visually ascertained by determining atrophy of the uterine horns at necropsy. One month after the OVX/Sham surgery, animals were subjected to unilateral ligature placement. The rats were then divided into four groups of 10 each: (1) Control (Sham + Vehicle), (2) Sham + Ligature + Vehicle, (3) OVX + Ligature + Vehicle, and (4) OVX + Ligature + Scl-Ab. To establish experimental periodontitis, 3/0 silk sutures (Johnson & Johnson Medical Ltd.; Shanghai, China) were placed subgingival to the right maxillary first and second molar teeth for 4 weeks with continuous "∞-ligature method". During this period, all four groups of rats received high-sugar drinking water (100 g/L) to facilitate the bacterial deposit and microbial biofilm formation. The ligature was checked twice weekly to ensure subgingival placement, and was replaced when necessary. Ovariectomies, sham operations and ligature placement were performed under 10% chloral hydrate general anesthesia administered intraperitoneally at 4 mL/kg. Rats from Control group were subjected to anesthesia to match initial ligature placement and OVX that was done in the other three groups.

Before starting Scl-Ab treatment, the ligatures were removed under the abovementioned general anesthesia. Vehicle (0.9% saline) or Scl-Ab (ratized monoclonal Scl-AbVI; Amgen Inc., Thousand Oaks, CA, USA) was administered subcutaneously at 25 mg/kg, twice weekly for 6 weeks. At the end of the study, all rats were sacrificed and their whole right maxilla specimens were harvested. The specimens were first fixed in 10% neutralized buffered formalin overnight (around 16 to 20 h) and then stored in 70% ethanol until the micro-CT scanning was performed. Whole blood was obtained by cardiac puncture at necropsy and then centrifuged to obtain serum. The serum was aliquot to 1 mL in individual sterile freezing tubes (Corning Incorporated, NY, USA) and stored at −80 °C until use. After micro-CT scanning was completed, right maxilla specimens were processed and embedded undecalcified in methyl methacrylate (MMA, M55909, Sigma, St. Louis, MO, USA). Sections were prepared from the occlusal surface of tooth crown to the alveolar bone mesiodistally along the plane parallel to the long axis of the teeth and then cut to 100–200 μm by Leica SP1600 Microtome (Leica, Heidelberg, Germany) and polished to a final thickness of approximately 20 μm by sequent usage of P300, P800 and P1200 sandpaper.

2.2. Micro-CT scanning and maxilla bone analysis

Right maxillary specimens were scanned using a cone beam micro-CT system (Skyscan1176; Skyscan, Kontich, Belgium) at Soochow University Orthopaedic Institute, Suzhou, China. The x-ray generator was operated at an accelerated potential of 50 kV with a beam current of 500 μA. The x-ray source 2-D detector operated with a shutter speed of 900 ms, which produces images with a voxel size of $9 \times 9 \times 9\ \mu m^3$. Scans were reconstructed, and three-dimensional digitized images were generated for each specimen using the supporting analyzing software (CT Analyser Version 1.10; Skyscan).

Linear measurement of the distance between the cemento-enamel junction (CEJ) and the alveolar bone crest (ABC) is used as an assessment for vertical ABL [8,19]. Vertical ABL is regarded as a hallmark of the

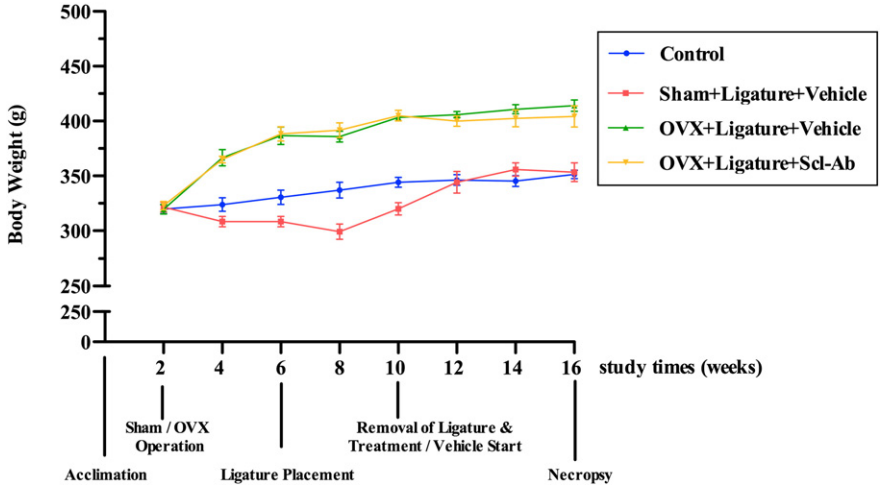

Fig. 1. Bi-weekly body weight record starting from OVX/Sham surgery until the end of the study. Mean ± SEM, $n = 15$. OVX, ovariectomized.