The effect of romosozumab on orthodontic tooth movement in ovariectomized rats

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The effect of romosozumab on orthodontic tooth movement in ovariectomized rats

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The Dissertation
Submitted to the Department of Dentistry
and the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Dental Science

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December 2023

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The Graduate School
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December 2023

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ABSTRACT

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Introduction

The demand for adult orthodontic treatment has consistently increased in recent years. Challenges in providing orthodontic treatment to the middle-aged population include periodontal issues and/or the impact of systemic diseases. In particular, osteoporosis, a systemic skeletal disorder characterized by decreased bone density and weakened bones, can greatly impact orthodontic treatment. Romosozumab, is a medication used to treat osteoporosis. It binds to sclerostin, thereby inhibiting its action. Romosozumab also exhibits a dual effect of initially promoting bone formation, and later, temporarily suppressing bone resorption. To date, the safety

and potential side effects of this medication in patients undergoing orthodontic treatment are unclear. As orthodontic treatment requires new bone formation, research in this area is warranted.

This study aimed to evaluate the effect of systemically administered sclerostin antibodies (Scl-Ab) on orthodontic tooth movements in an ovariectomized osteoporotic animal model.

Materials and Methods

Twenty-four 12-week-old female Sprague-Dawley rats were randomly divided into two groups: 1) OVX (ovariectomy) group, 2) ROMO (ovariectomy + romosozumab) group. Ovariectomy was performed for all 24 rats, followed by an 8-week waiting period. The ROMO group received subcutaneous injections of romosozumab twice a week, starting two weeks after ovariectomy, while the OVX group received an equivalent amount of saline. Eight weeks after the ovariectomy, an orthodontic force of 50g was measured and applied by connecting orthodontic elastic bands between the maxillary first molar and a mini-screw to facilitate tooth movement (orthodontic treatment). Subsequently, the rats were sacrificed on days 5, 7, 10, and 14. Impressions were taken before the experiment and before sacrificing the rats. Plaster models were made from these impressions and scanned to measure the amount of tooth movement. The effects on periodontal tissues were

evaluated through micro-computed tomography (micro-CT) analysis, tartrate-resistant acid phosphatase (TRAP) staining, and immunohistochemistry (IHC) analysis.

Results

The ROMO group showed more tooth movement on the 7th day of orthodontic treatment. Conversely, on the 10th and 14th days, relatively less movement was observed. Analysis of the root furcation area of the maxillary first molars revealed that from the 7th day, bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th) significantly increased in the ROMO group, while trabecular separation (Tb.Sp) significantly decreased. More TRAP-positive cells were observed in the compression side of the OVX group, the ROMO group exhibited a marked decrease in the positive expression of the nuclear factor-kappa β ligand (RANKL) receptor-activator, osteoprotegerin (OPG), and sclerostin. The OPG/RANKL ratio demonstrated marked expression differences between the two groups. The ROMO group exhibited a higher ratio than the OVX group, and the tension side exhibited a higher ratio than the compression side demonstrating significant differences.

Conclusions

Romosozumab initially accelerated tooth movements, but later inhibited tooth movement. As new alveolar bone is formed, the micro-CT parameters are also improved. Importantly, in the ROMO group, osteoclasts, bone remodeling markers, and sclerostin decreased, while the OPG/RANKL ratio became higher.

Key words: osteoporosis; osteoporosis medication; anti-sclerostin antibody; Wnt signaling pathway; orthodontic treatment

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I. INTRODUCTION

Orthodontic tooth movement (OTM) is a complex process that results in physiological changes in teeth and surrounding tissues due to mechanical force.[1] Bone resorption in this process is mediated by osteoclasts and cells of the monocyte/macrophage lineage, while osteoclastogenesis is regulated by the nuclear factor kappa B ligand (RANKL) receptor-activator expressed by cells surrounding the tooth root. Bone remodeling refers to the biological process in which osteoclasts and osteoblasts, functionally balance each other in the simultaneous processes of bone destruction and formation. The interaction between osteocytes, osteoblasts, and osteoclasts leads to changes in the periodontium including the cementum,

periodontal ligament (PDL), alveolar bone, and gingiva.[2] In particular, the PDL is a complex connective tissue composed of fibroblasts that is sensitive to mechanical loading and serves important functions, such as supplying blood and nutrients to surrounding tissues and transmitting and absorbing mechanical stress.[3] The PDL and alveolar bone undergo remodeling in response to orthodontic forces. This occurs on both the compression and tension sides of the structures. On the tension side, a different pattern emerges, with an increased expression of osteoprotegerin (OPG), a decoy receptor for RANKL. An increase in OPG interferes with osteoclast formation and promotes the formation of new bone.

Orthodontic treatment is common among children and adolescents. In recent years, the proportion of adults undergoing orthodontic treatment has also increased steadily[4] according to the American Association of Orthodontists. In particular, middle-aged individuals are showing a growing interest and greater demand for orthodontic treatment as a way to improve their quality of life and enhance aesthetics.[5,6] Some may also seek to improve dental health and function through orthodontic treatment. Caution is warranted when providing orthodontic treatment to middle-aged adults, as periodontal issues and/or underlying systemic conditions are common. Aging is often accompanied by a greater risk of systemic diseases,

which should not be overlooked during orthodontic treatment. Of the systemic conditions common in mid-life, osteoporosis is of significant concern.

Osteoporosis causes low bone mass, decreased bone strength, and poor microstructure, which in turn, leads to increased fracture risk and greater bone fragility.[7] Osteoporosis is managed according to general guidelines or the use of osteoporosis medications. Osteoporosis medications can be broadly categorized into two groups according to the mechanism of action. Anti-resorptive drugs (ARDs), such as bisphonsphonates and denosumab, work by inhibiting osteoclastic activities, and thereby preventing bone resorption, while anabolic agents, such as parathyroid hormone (PTH) and romosozumab, activate osteoblasts to promote bone formation. Moreover, anabolic agents are known for their pronounced effect on increasing bone mass relative to ARDs. Generally, anabolic agents are used as first-line treatment for osteoporosis, especially in patients at very high risk of osteoporosis.

Recently, romosozumab (Amgen Inc., Thousand Oaks, CA, USA, and UCB Brussels, Belgium), a neutralizing monoclonal antibody against the Wnt inhibitor sclerostin, has been introduced as a treatment for osteoporosis. Romosozumab is a human immunoglobulin monoclonal antibody that binds to sclerostin to inhibit its

action. It is the first dual-effect anabolic medication available for the management of osteoporosis. Romosozumab initially promotes bone formation, and then, temporarily suppresses bone resorption later by modulating RANKL and OPG.[8] It is particularly useful for treating osteoporosis in elderly women.[8,9] Sclerostin, which inhibits bone formation, is a potential new target for osteoporosis treatment and has gained attention as an essential regulatory factor in both bone formation and resorption within the canonical Wnt signaling pathway.[10] However, research in this area is lacking, as there is currently insufficient data regarding the safety and potential side effects of using these new medications during orthodontic treatment, especially when new bone formation is desirable. Osteoporosis medications have distinct mechanisms of action. Some patients receiving these medications may also require orthodontic treatment. Therefore, it is crucial to investigate the potential interactions between these medications and orthodontic procedures.

This study aimed to investigate the effects of systemically administered sclerostin antibodies (Scl-Ab) on OTM and the associated molecular mechanisms, in an osteoporosis animal model. We hypothesized that Scl-Ab, by modulating the expression of RANKL, OPG, and sclerostin, would initially increase OTM, and subsequently decrease it.

II. MATERIALS AND METHODS

2.1. Animals

This experiment involved twenty-four 12-week-old female Sprague-Dawley rats, with a mean weight of 274g. The selection, management, and surgical preparation protocols were conducted according to the standards approved by the Institutional Animal Care and Use Committee of Yonsei Medical Center, Seoul, Korea (AICUC 2020-0010). The animals underwent a one-week acclimation period and were housed in cages with a 12-hour light-dark cycle, at a temperature of 20°C ± 5°C, and humidity of 50% ± 10%. They were provided with water and a standard laboratory pellet diet.

2.2. Experimental procedures

2.2.1. Ovariectomy

All 24 rats underwent surgery at 12 weeks of age. Bilateral ovariectomy was performed under general anesthesia using a combination of intraperitoneal Zoletil® (tiletamine and zolazepam, 50 mg/ml, 0.6 ml/kg body mass; Virbac lab. Carros, France) and Rompun® (zylazine, 23.32 mg/ml, 0.4 ml/kg body mass; Bayer, Leverkusen, Germany). First, the fur in the surgical area was removed, and the area was disinfected. Second, a 1 cm incision was made on the dorsal side. Third, the ovaries were located and removed, and the incision sites of the oviduct were ligated. Finally, the surgical incision site was sutured using vicryl 4-0 sutures. After surgery, subcutaneous meloxicam (1 mg/kg, once a day for 3 days; Metacam®, Boehringer Ingelheim, Rhein, Germany) and enrofloxacin (10 mg/kg/day, once a day for 3 days; Baytril®, Bayer, Germany) were administered. Subsequently, the rats were weighed weekly (Figure 1).

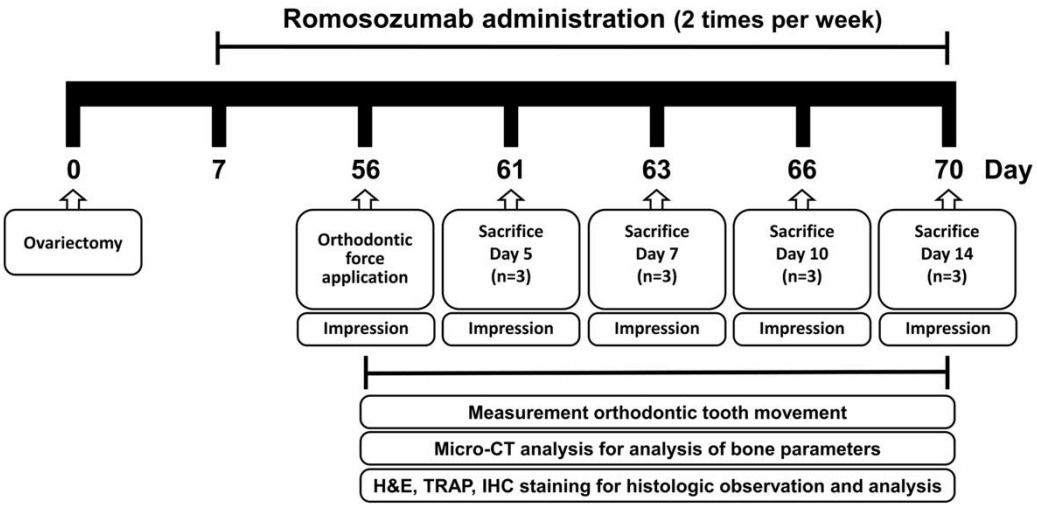


Figure 1. The experimental design

2.2.2. Medication

The rats were randomly divided into two groups of 12 rats: the ovariectomized (OVX) group and the ovariectomized + romosozumab (ROMO) group. Two weeks after the ovariectomy surgery, the ROMO group received subcutaneous injections of romosozumab (Evenity®) at a dose of 25 mg/kg twice a week, while the OVX group received vehicle injections only until the time of sacrifice.

2.2.3. Orthodontic tooth movement

In this study, tooth movement was induced in the rats 8 weeks after ovariectomy. To apply the orthodontic appliance, a 6 mm mini-screw (Wezendental, Ø 1.6mm, Seoul, Korea) was inserted in the outer alveolar bone region of the maxillary anterior teeth. A .008-stainless steel ligature wire was fixed to the maxillary first molar and connected to the mini-screw using an orthodontic power chain (Generation II, Ormco, USA). Approximately 50g of force was measured using a pull gauge and the elastic band was attached to the mini-screw (Figure 2). This procedure was performed on both the left and right sides of each rat in both groups. To maintain a consistent force, the power chain was replaced on days 5 and 10. The rats were sacrificed on days 5, 7, 10, and 14 for further analysis.

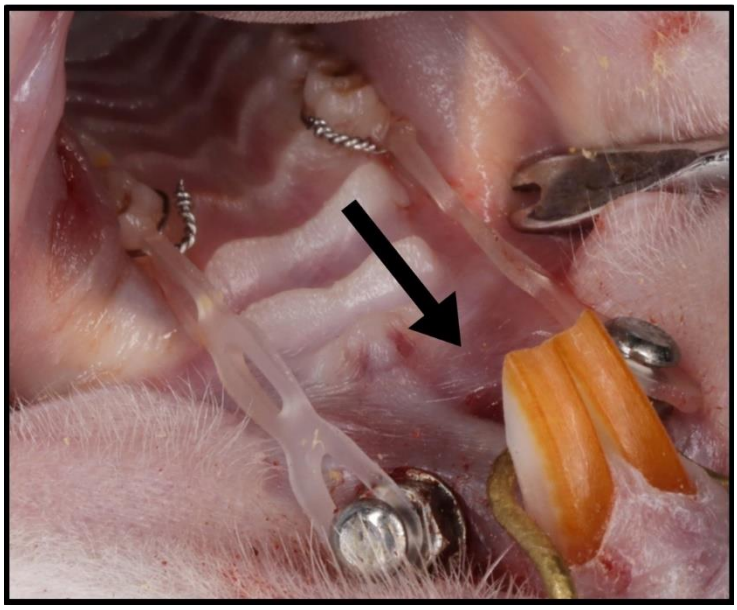


Figure 2. Orthodontic tooth movement model in rats

The black arrow shows the direction of the force.

2.3. Distance of orthodontic tooth movement

Before applying the orthodontic power chain, impressions of the maxillary teeth were taken using polyvinylsiloxane impression material (Aquasil Ultra XLV, Dentsply DeTrey GmbH, Konstanz, Germany), and impressions were taken again on days 5, 7, 10, and 14. Dental stone (Neo super plumstone, Mutsumi, Japan) was used to create plaster models from the impressions, which were then scanned using the Identica hybrid 3D dental laser scanner (MEDIT T510, Seoul, Korea). The scanned data was provided in standard tessellation language (STL) file format. The OTM distance was measured using the same software (Meshmixer, Autodesk Inc, America). The OTM distance was measured as the distance from the mesial part of the maxillary first molar to the distal part of the maxillary third molar. The difference between the post-experiment and pre-experiment measurements was recorded.

2.4. Micro-computed tomography (Micro-CT) analysis

After resecting the maxillary molars, along with adjacent alveolar bone and surrounding tissues, the specimens were fixed in 10% formalin for one week. Subsequently, they were imaged using a Skyscan 1173 micro-CT scanner (Bruker-CT, Kontich, Belgium) under the following conditions: pixel size of 15 μm, voltage of 130 kV, current of 60 μA, and a 1.0 mm aluminum filter. The captured images were then reconstructed and analyzed using CTAn software (Bruker-CT, Kontich, Belgium). A region of interest (ROI) was selected in the furcation area of the maxillary first molar, with dimensions of 400 μm × 400 μm × 500 μm (length × width × thickness, in the sagittal plane) (Figure 3). Care was taken to ensure that the measurement did not extend beyond the tooth roots and non-bony structures, as the shape of the furcation area may vary slightly among animals. From the extracted data, bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) were analyzed. Three different regions were selected for each specimen, and the average values were calculated.[11,12] The definitions of various bone parameters are as follows.

- BV/TV: Represents the ratio of trabecular bone volume to the total bone volume.
- Tb.Th: Measures the average thickness of trabeculae within voxels representing bone using direct three-dimensional (3D) methods.
- Tb.N: Measures the average number of trabeculae per unit length.
- Tb.Sp: Represents the average distance between trabeculae, measured using direct 3D methods.

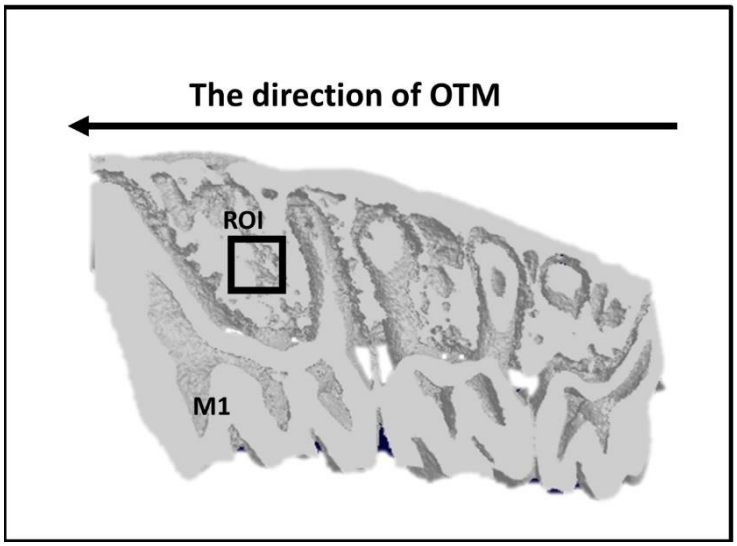


Figure 3. Three-dimensional micro-CT analysis

The black arrow indicates the direction of orthodontic tooth movement. The black square frame indicates the region of interest. OTM, orthodontic tooth movement; M1, maxillary first molar.

2.5. Histological analysis

2.5.1. Hematoxylin and eosin (H&E) staining

After Micro-CT analysis, all samples were decalcified with 10% EDTA (pH 7) and dehydrated before being embedded in paraffin. The cut plane was selected along the axial axis, from the cervical to the apical region of the tooth, and serially sliced into 3 μm thickness, ensuring a satisfactory section. These sections were then stained with hematoxylin and eosin (H&E). Among the five roots of the maxillary first molars in the sample, the mesial root, which is the largest and provides better visualization of the root structure, was selected for histological evaluation.

2.5.2. Tartrate-resistant acid phosphatase (TRAP) staining

To evaluate TRAP expression, paraffin blocks of 5 μm thickness were subjected to TRAP staining using a commercial kit (Sigma, St. Louis, MO, USA). Following the manufacturer's protocol, the pre-warmed slides were treated with the TRAP staining solution mix and incubated at 37°C for 30-60 minutes. TRAP-positive cells were stained with the TRAP staining solution, and nuclei were counterstained with hematoxylin solution. Osteoclasts were classified as satisfying the conditions of TRAP-positive, multinucleated cells located along the bone surface. Three randomly selected sections from the periodontal ligament (PDL) of the maxillary

first molar's pressure side were measured and presented as mean ± standard deviation (SD) values.[13]

2.5.3. Immunohistochemical (IHC) analysis

For immunohistochemical (IHC) staining, paraffin blocks were cut into 3 μm thickness. All IHC staining procedures from deparaffinization to counterstaining were performed using an automated Ventana Discovery XT staining instrument (Ventana Medical Systems, Inc., Tucson, AZ, USA). Mouse anti-RANKL monoclonal antibody (1:6400 dilution; sc-377079, Santa Cruz, USA), rabbit anti-OPG polyclonal antibody (1:400 dilution; ab203061, Abcam, USA), and rabbit anti-sclerostin polyclonal antibody (1:400 dilution; ab85799, Abcam, USA) were used as primary antibodies. Secondary antibodies were applied according to the manufacturer's recommendations. Subsequently, the sections were visualized using a diaminobenzidine (DAB) coloration kit and counterstained with hematoxylin. The IHC staining intensity was measured using the IHC profiler plugin in ImageJ software (version 1.53, National Institutes of Health, USA) after magnifying the ROI in the gingival area by 200x.[14] The plugin's color deconvolution method was used to digitally separate DAB and hematoxylin nuclear staining and calculate their respective contribution. After deconvolution, the DAB image was converted to an 8-bit format, and a threshold value was set to consider black pixels as positive DAB

staining. The stained RANKL, OPG, and sclerostin were quantified based on the values of black pixels in the respective ROIs and the overall mean areas were compared between groups.[15]

All histological stained slides were scanned using the Aperio AT2 image-capturing device (Leica Biosystems, Wetzlar, Germany), and evaluated at x200 and x400 magnification using Imagescope software 12.3 (Aperio Technologies, Vista, CA, USA). The stained slides were measured by a single- blinded researcher.

2.6. Statistics

This study was analyzed using SPSS Version 23.0 (Statistical Package for the Social Sciences, IBM, USA) at a 95% confidence level. All data were presented as mean ± standard deviation (SD). An independent t-test was used to analyze the distance of OTM and micro-CT parameters. For analyzing the number of TRAP-positive cells and the expression levels of RANKL, osteoprotegerin, sclerostin, and the ratio of OPG and RANKL, the Mann-Whitney test was employed.

III. RESULTS

3.1. The mean body weight of the rats

The body weight of the rats was measured weekly. During the osteoporosis induction period after ovariectomy, the mean body weight increased in a normal pattern. One week after orthodontic devices were placed for OTM, there was a slight decrease in the mean body weight.

3.2. The distance of tooth movement

The amounts of OTM are shown in Figure 4. When comparing the values between the OVX group and the ROMO group, a statistically significant difference was observed. The measurements recorded on day 7, day 10 and day 14 showed a significant difference between the two groups. The amount of OTM in the OVX group increased over the experimental period. In contrast, in the ROMO group, the amount of OTM increased until day 7, and then, an inhibiting tendency was observed from day 10.

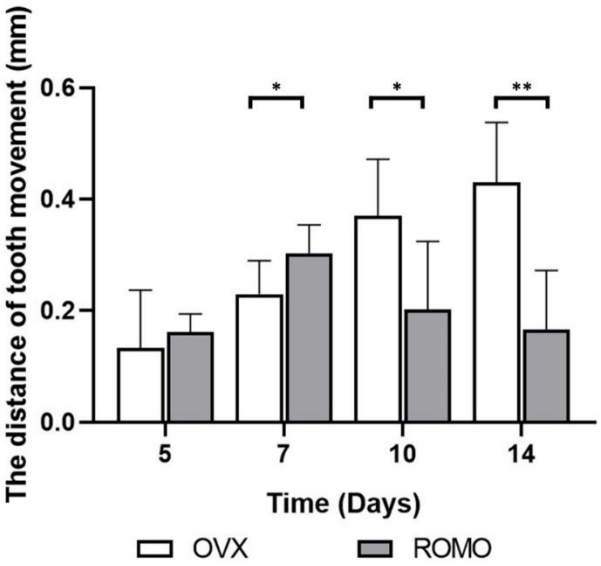


Figure 4. The distance of orthodontic tooth movement on days 5, 7, 10 and 14

Data are presented as the mean ± standard deviation. * and ** indicate P<0.05 and P <0.01, respectively.

3.3. Micro-computed tomographic (micro-CT) analysis

The analysis of the furcation area of the maxillary first molar showed that romosozumab increased BV/TV, BV/Th, and Tb.N, and decreased Tb.Sp in the ovariectomized rats, with significant differences observed in all parameters.

When comparing the values between the OVX group and the ROMO group, a statistically significant difference was observed in all parameters on the 7, 10, and 14 days (Figure 5).

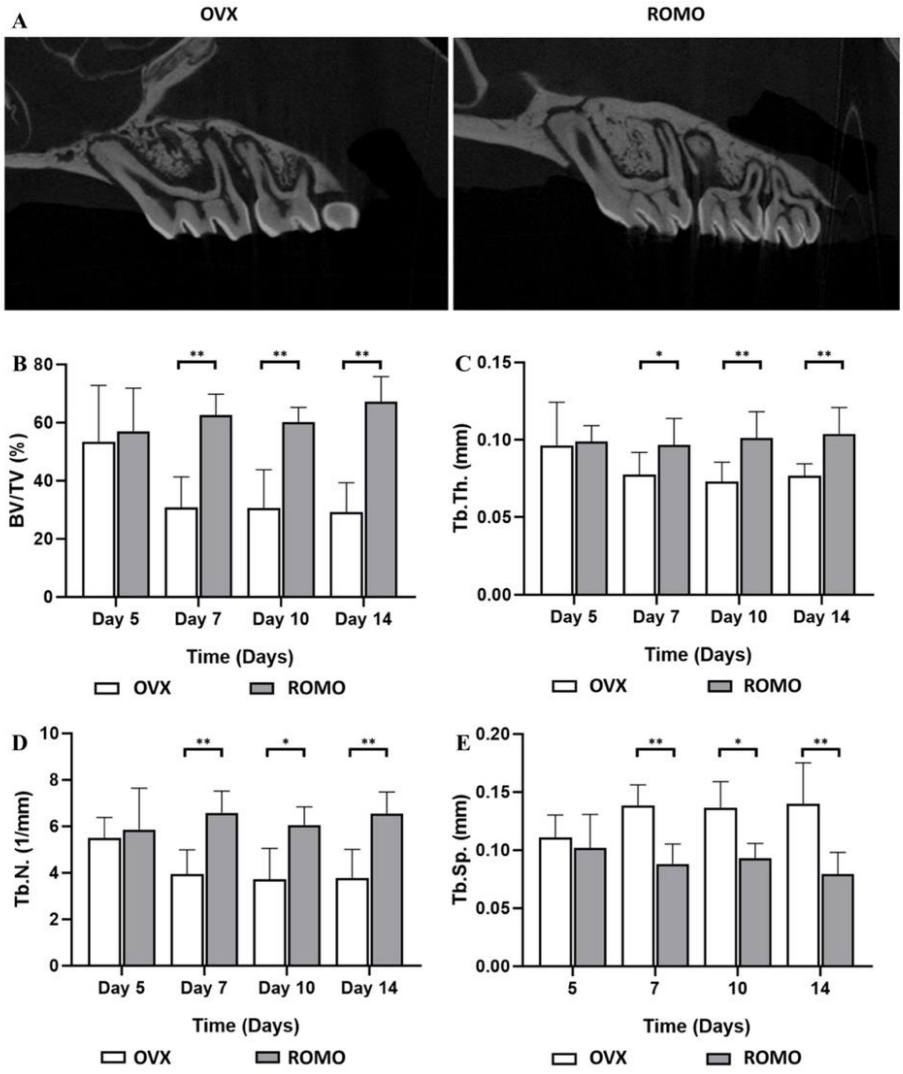


Figure 5. Micro-CT analysis of furcation area of the maxillary first molar

(A) Micro-CT scan of the maxillae in the control and experimental groups, (B) Micro-CT anlalysis of BV/TV. (C) Micro-CT analysis of Tb.Th. (D) Micro-CT analysis of Tb.N. (E) Micro-CT analysis of Tb.Sp. * and ** indicate P <0.05 and P <0.01, respectively.

3.4. Clinical observations

For schematic observation, the mesial root of each maxillary first molar was sectioned in the horizontal plane and stained with H&E. Inflammation was not observed in either of the two groups. Conversely, blood vessels and Howship's lacunae were found on the compression side in both groups.

3.5. Histological analysis

To quantify the osteoclasts, TRAP staining was performed. TRAP-positive multinucleated cells observed on the compression and tension sides of each maxillary first molar were counted (Figures 6 and 7). The OVX group showed a higher number of TRAP-positive cells on both the compression and tension sides compared to the ROMO group, indicating significant differences between the groups at all time points.

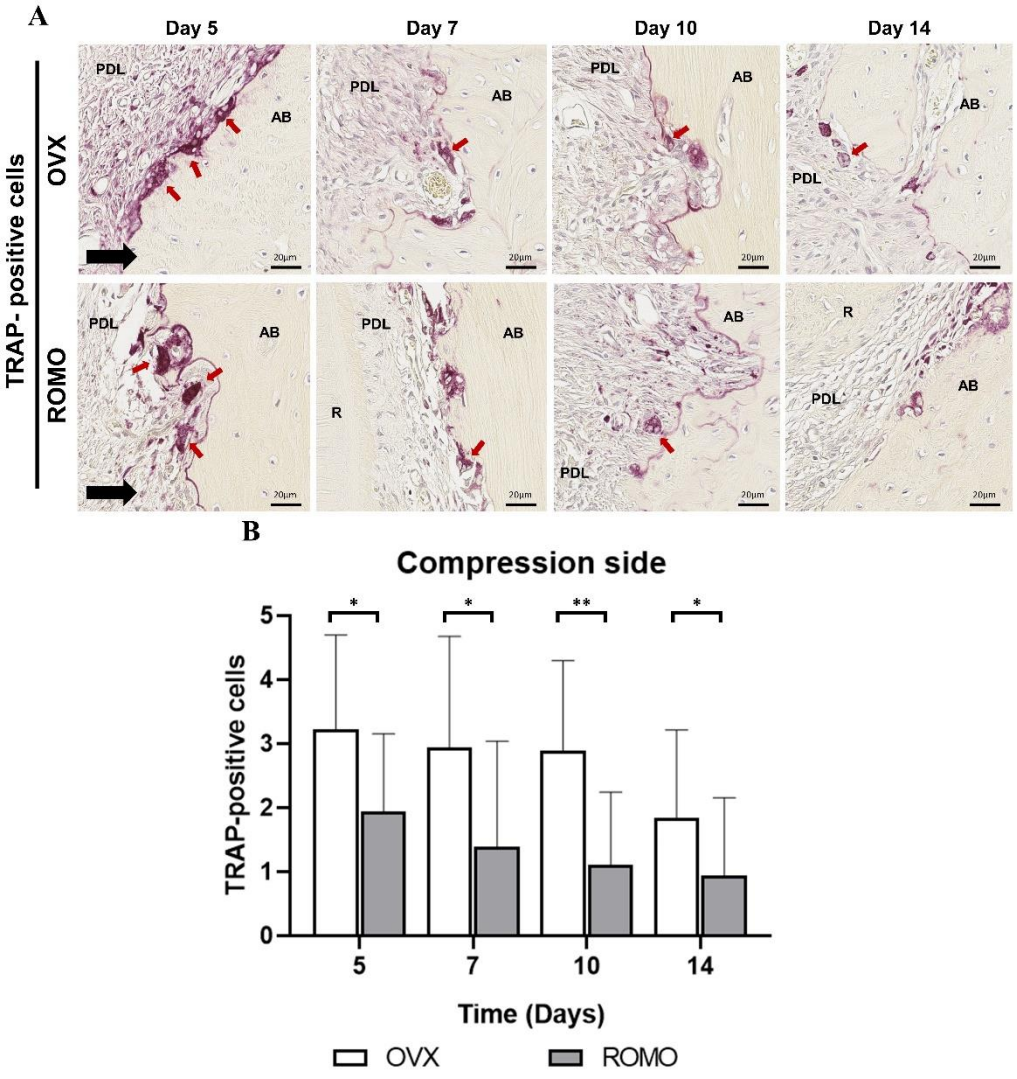


Figure 6. TRAP staining of maxillary first molar at the compression side

(A) The TRAP staining on the compression side. (B) The number of TRAP-positive osteoclasts on the compression side.

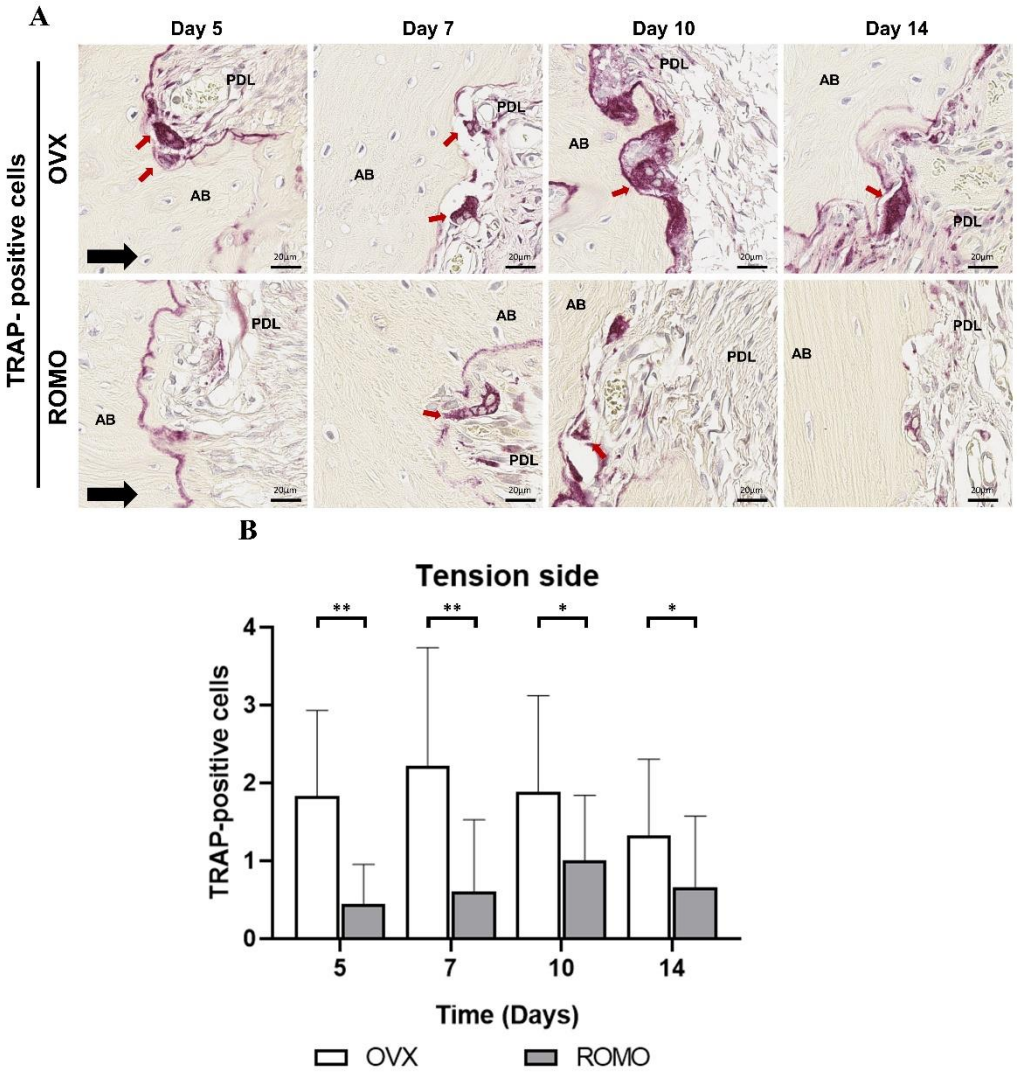


Figure 7. TRAP staining of maxillary first molar at the tension side

(A) The TRAP staining on the tension side. (B) The number of TRAP-positive osteoclasts on the tension side.

RANKL, OPG, and sclerostin expression were visualized by DAB substrate, and their presence was observed in the mesial root of each maxillary first molar, as indicated by a brownish-yellow color. RANKL and OPG were observed in the PDL cells. Immunohistochemically, the ROMO group showed lower positive expression of RANKL on both the compression and tension sides compared to the OVX group, demonstrating significant differences between the groups at all time points (Figures 8 and 9). The ROMO group also showed lower levels of OPG positive expression on both sides, with significant differences at all time points (Figures 10 and 11). Sclerostin was observed in the osteocytes of the alveolar bone. Importantly, a decrease in sclerostin was confirmed at all time points in rats administered with romosozumab. After the administration of romosozumab in ovariectomized rats, there was a marked decrease in the positive expression of RANKL, osteoprotegerin, and sclerostin on both the compression and tension sides (Figures 12 and 13).

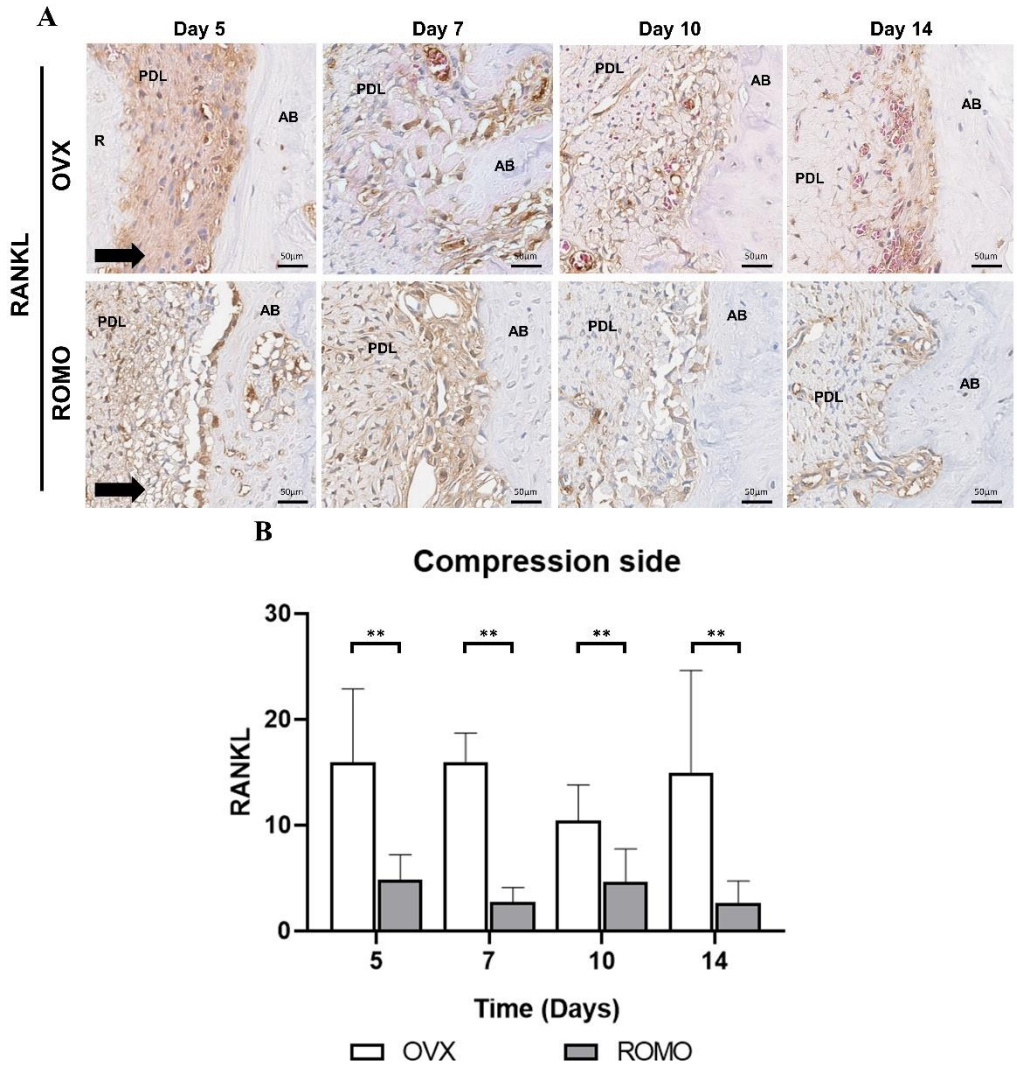


Figure 8. Immunohistochemical staining of RANKL at the compression side

(A) The IHC staining of RANKL at the compression side. (B) The positive expression of RANKL. AB, alveolar bone; PDL, periodontal ligament; R, root; D, dentin. * indicates P <0.05 and ** indicates P <0.01.

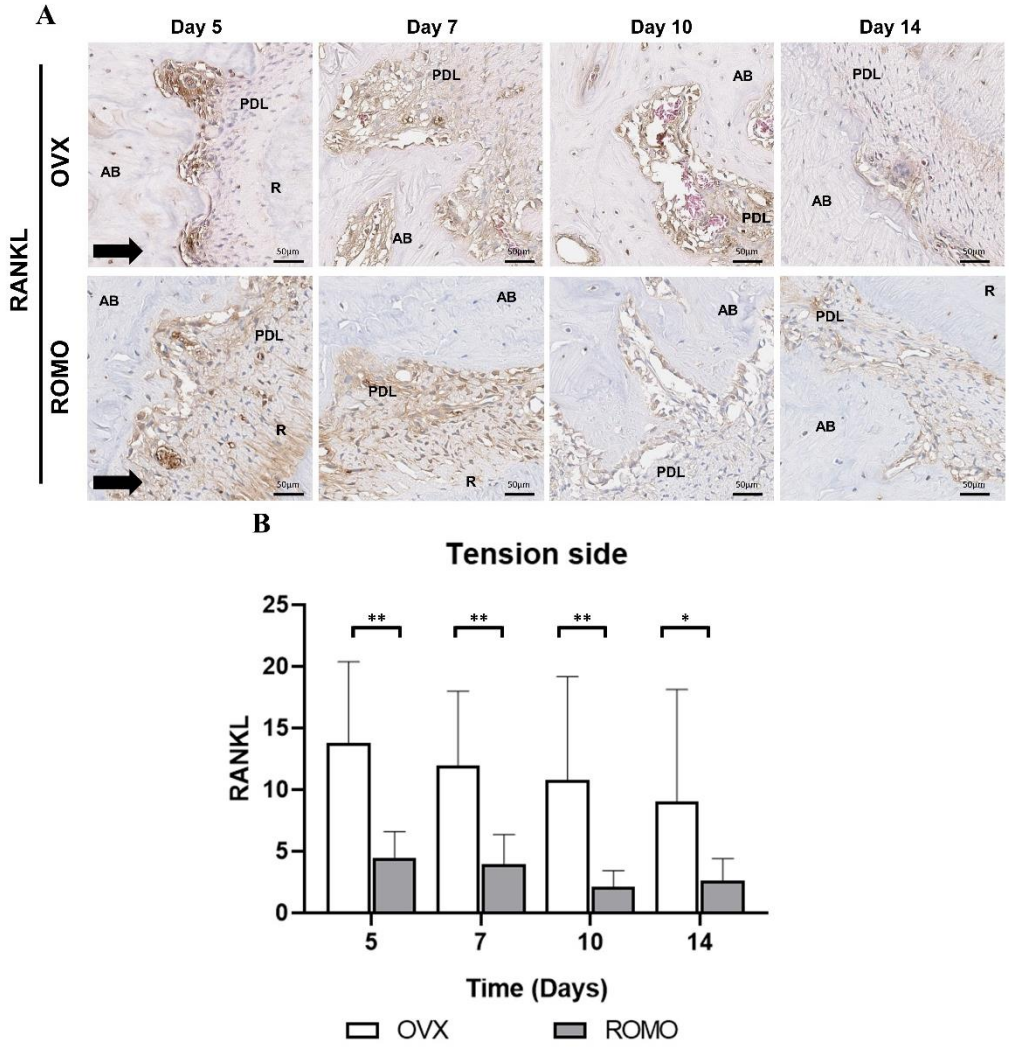


Figure 9. Immunohistochemical staining of RANKL at the tension side

(A) The IHC staining of RANKL at the tension side. (B) The positive expression of RANKL. AB, alveolar bone; PDL, periodontal ligament; R, root; D, dentin. * indicates P <0.05 and ** indicates P <0.01.

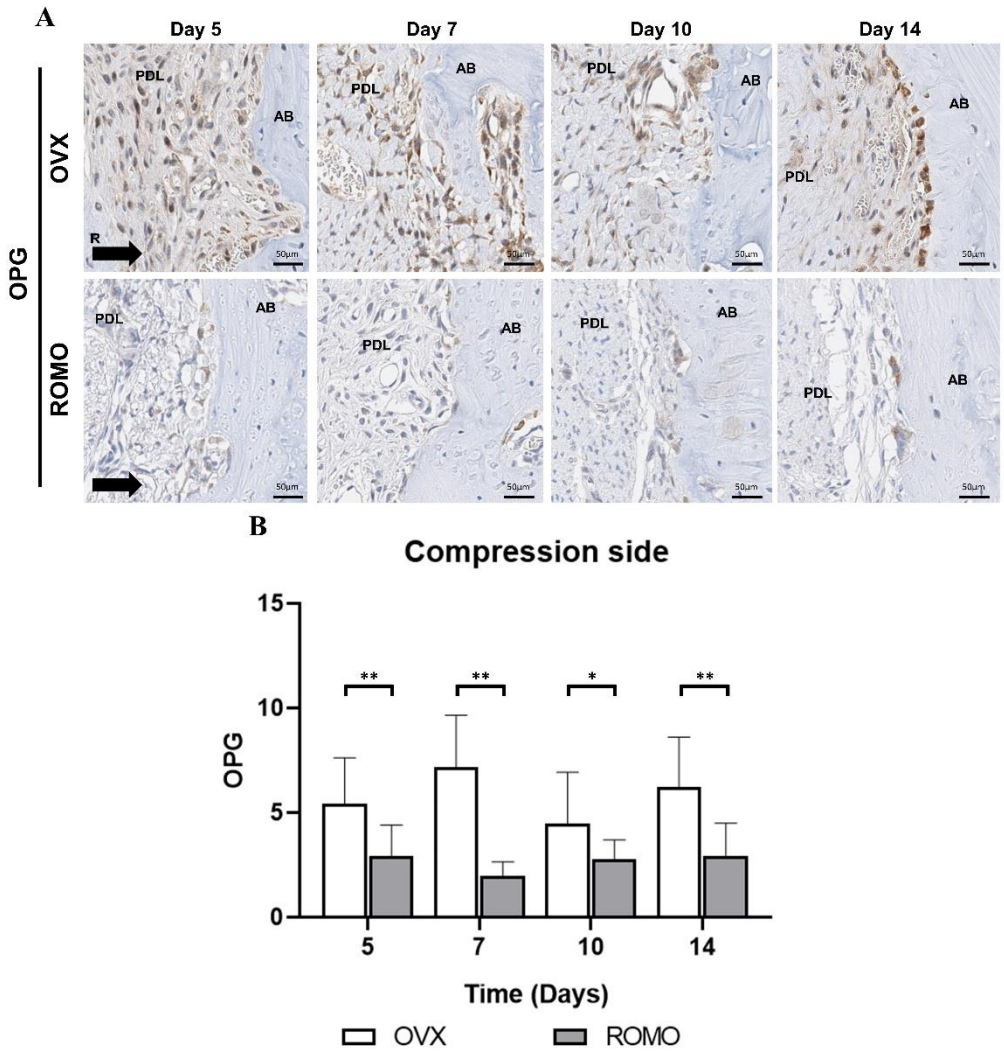


Figure 10. Immunohistochemical staining of OPG at the compression side

(A) The IHC staining of OPG at the compression side. (B) The positive expression of OPG. AB, alveolar bone; PDL, periodontal ligament; R, root; D, dentin. * indicates P <0.05 and ** indicates P <0.01.

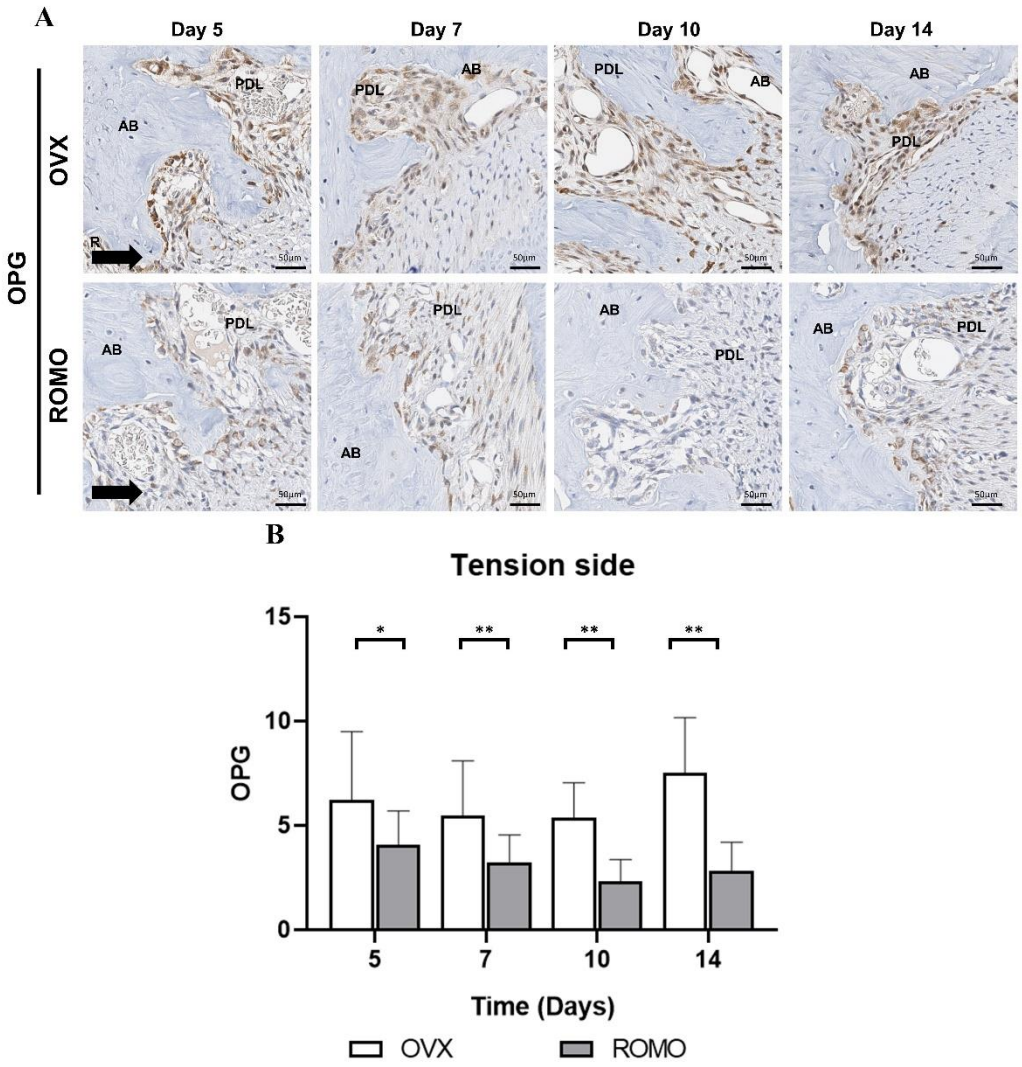


Figure 11. Immunohistochemical staining of OPG at the tension side

(A) The IHC staining of OPG at the tension side. (B) The positive expression of OPG. AB, alveolar bone; PDL, periodontal ligament; R, root; D, dentin. * indicates $P < 0.05$ and ** indicates $P < 0.01$.

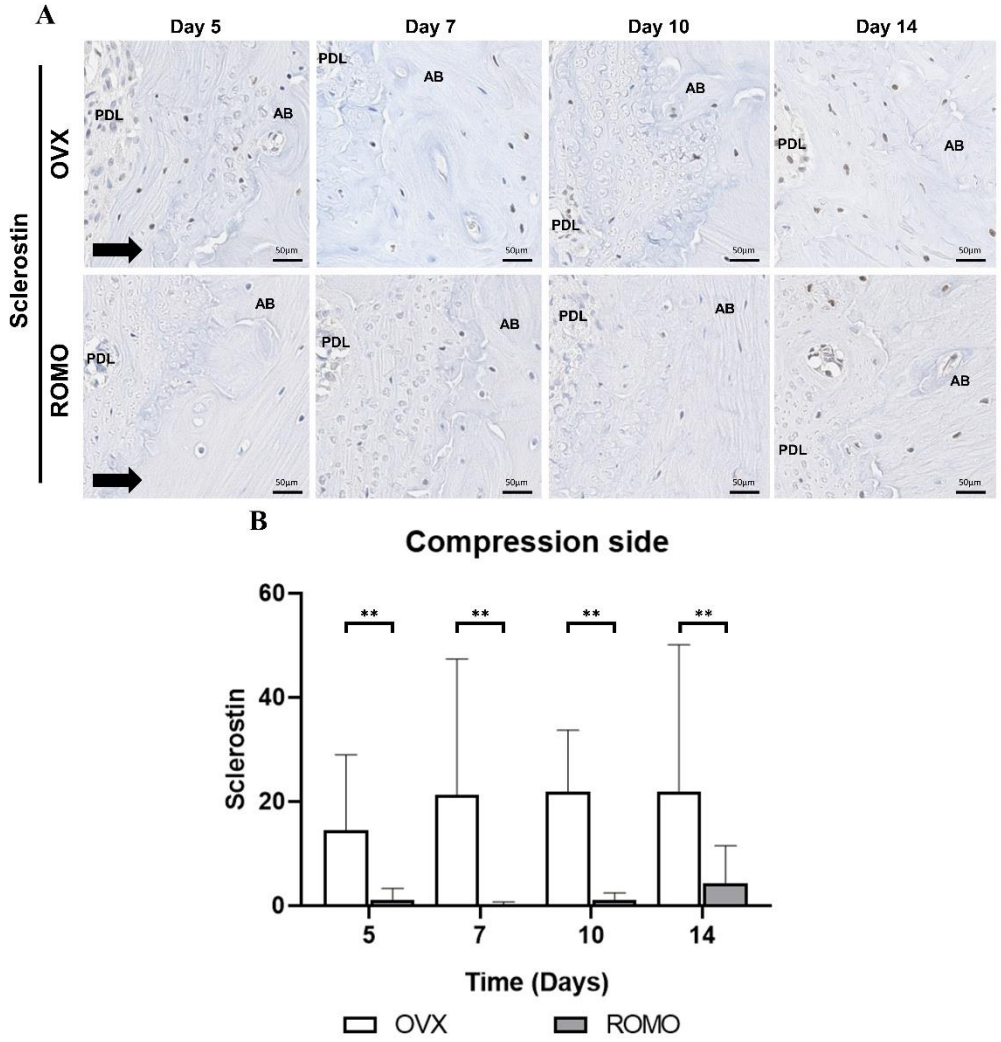


Figure 12. Immunohistochemical staining of sclerostin at the compression side

(A) The IHC staining of sclerostin at the compression side. (B) The positive expression of sclerostin. AB, alveolar bone; PDL, periodontal ligament; R, root; D, dentin. * indicates P <0.05 and ** indicates P <0.01.

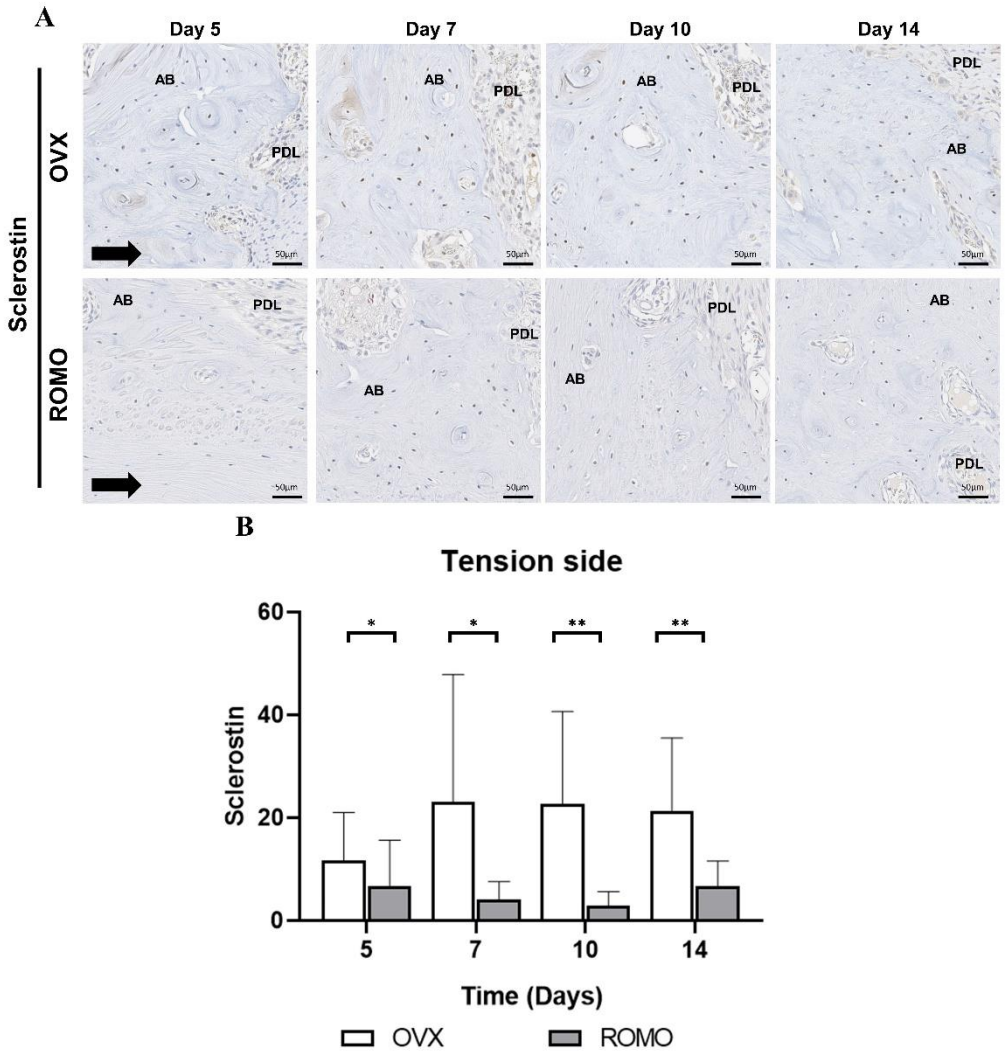


Figure 13 .Immunohistochemical staining of sclerostin at the tension side

(A) The IHC staining of sclerostin at the tension side. (B) The positive expression of sclerostin. AB, alveolar bone; PDL, periodontal ligament; R, root; D, dentin. * indicates P <0.05 and ** indicates P <0.01.

From an immunohistochemical perspective, the OPG/RANKL ratio demonstrated marked expression differences between the two groups. On the compression side, the ROMO group exhibited a higher ratio than the OVX group, demonstrating statistically significant differences at all time points (Figure 14). On the tension side, the ROMO group showed a higher ratio on days 5 and 7. A difference between the compression and tension sides was also observed. According to quantitative data, the OPG/RANKL ratio was higher on the tension side than on the compression side in both the OVX and ROMO groups (Figure 15).

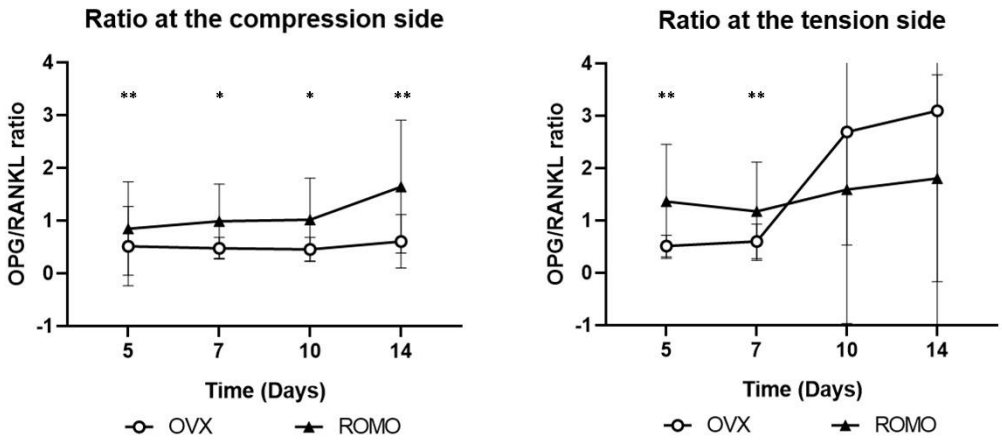


Figure 14. The ratio of OPG/RANKL according to group

OVX (ovariectomy group), ROMO (ovariectomy + romosozumab group).

* indicates P <0.05 and ** indicates P <0.01.

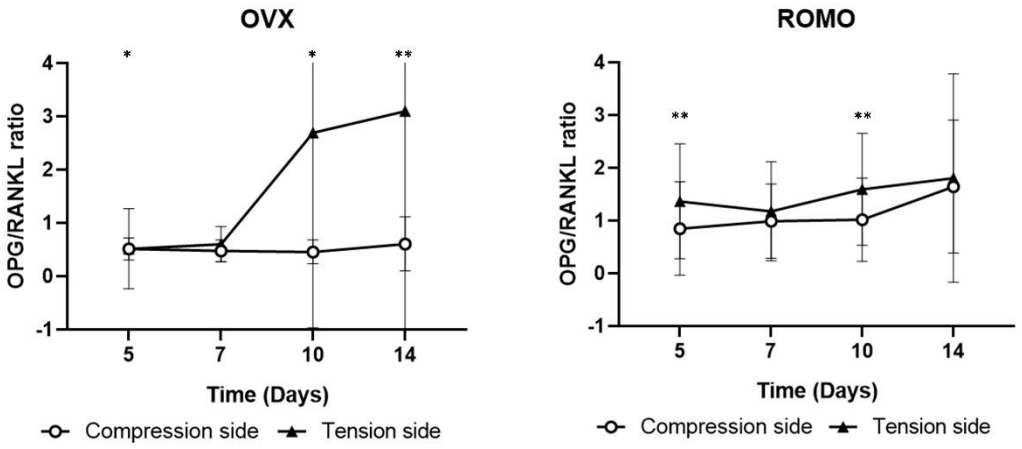


Figure 15. The ratio of OPG/RANKL according to compression and tension side

OVX (ovariectomy group), ROMO (ovariectomy + romosozumab group).

* indicates P <0.05 and ** indicates P <0.01.

IV. DISCUSSION

4.1. Considerations for orthodontic treatment in middle-aged adults

OTM is dependent on various factors including the magnitude of the force, speed, and distance, which in turn, influence alveolar bone remodeling. Moreover, there are physiological differences between adults and children or adolescents, which further impact treatment outcomes. This is because adults have a limited number of progenitor cells, a decreased density of fibroblasts, and reduced vascular formation capabilities. These reduce bone turnover, leading to decreased flexibility and elasticity of the alveolar bone.[16] Similarly, the PDL also experiences reduced cellular and metabolic activities with advancing age.[17] Additionally, the proliferative capacity of osteoblasts decreases, further reducing bone formation abilities.[16] Overall, aging leads to functional deterioration in all body tissues, resulting in weaker bones and compromised mechanical functions.[18,19] Considering these, the patient's age is one of the factors that determine the periodontal conditions during OTM.

4.2 The relationship between osteoporosis and orthodontic treatment

4.2.1 Characteristics of Osteoporosis

Osteoporosis is a systemic skeletal disorder characterized by a reduction in bone mineral density (BMD), resulting in weakened bone strength and increased fracture risks. Bone remodeling is disrupted due to an increased rate of bone resorption relative to bone formation. As osteoporosis progresses, not only does the thickness of cortical bone decrease, but also the microarchitecture of trabecular bone is disrupted, affecting bone strength and increasing fracture risks. Given the rapidly aging population in South Korea, osteoporosis, a representative age-related systemic disease, has become a significant societal issue. Osteoporosis is not always detected because it is associated with non-specific symptoms, which may lead to delays in seeking medical attention. Once a fracture occurs, there is a higher risk of subsequent or new fractures. Patients with osteoporosis may be broadly classified into two categories: those with primary osteoporosis due to menopause and aging, and those with secondary osteoporosis resulting from specific diseases, surgeries, or medications. Postmenopausal women with primary osteoporosis often experience a significant decrease in PTH secretion due to estrogen deficiency, leading to a reduction in vitamin D and calcium absorption. This ultimately results in a decrease in bone mass. Moreover, estrogen deficiency increases osteoclastic activities, bone resorption, and reduced bone mass and bone density, resulting in

bone deterioration and an increased risk of fractures. Currently, medications, such as bisphosphonates, denosumab, PTH, and romosozumab, are used to treat osteoporosis. Since the medications have different mechanisms of action, patients may experience diverse effects and also different side effects.

4.2.2. Orthodontic treatment in patients with osteoporosis

For patients with osteoporosis who undergo orthodontic treatment, the rate of OTM tends to accelerate, resulting in greater tooth displacement and an increased risk of relapse. [6,20] Consequently, the effects and impacts of applying orthodontic forces to patients with osteoporosis must be considered. Previous research has reported that applying OTM to rats with ovariectomy-induced osteoporosis increases tooth displacement and osteoclast numbers, thus, affecting the speed of OTM.[21,22] These results indicate that when there is significant alveolar bone resorption due to osteoporosis, teeth can move excessively beyond the intended amount during orthodontic treatment, leading to potentially harmful effects. This phenomenon can be attributed to an imbalance in bone remodeling due to a decreased bone formation capacity.

4.2.3 Osteoporosis medication and orthodontic treatment

Providing orthodontic treatment to patients with osteoporosis who are on medication therapy must also be considered. The fundamental principle of orthodontic treatment involves tooth movements within the alveolar bone in response to applied mechanical forces. Various factors including age and medications, can potentially influence tooth movement. Several studies have reported on the effects of medication on orthodontic treatment in patients with osteoporosis.[23,24] For example, bisphosphonates, which interfere with osteoclast activity, can inhibit tooth movement, thus, potentially prolonging the orthodontic treatment duration. Currently, various osteoporosis medications are available. Each medication has its distinct mechanisms of action, providing different effects, and associated with different side effects. Consequently, the relationship between orthodontic treatment and osteoporosis medications must be considered.

4.3 Osteoporosis medications

4.3.1. Introduction and description of the latest osteoporosis medications

The expected benefits of osteoporosis medications include reducing bone loss and increasing bone mineral density, thereby decreasing fracture risks. The goal of orthodontic treatment in patients with osteoporosis is also to prevent fractures through appropriate tooth movement, while completing the orthodontic treatment. Effective orthodontic treatment planning must consider the patient's medications. There are two main categories of osteoporosis medications: ARDs and anabolic agents (Table 1).

Table 1. Osteoporosis medications characteristics

Medication type	Medication		Features
ARD	Bisphosphonate	Alendronate Zoledronate Risedronate Clodronate Pamidronate	· Inhibits differentiation and function of osteoclasts · Resides in the bone for ten years · Exhibits the most potent inhibition of bone resorption · The most frequently prescribed
Anabolic agent	Denosumab	Prolia Xgeva	· RANKL monoclonal antibody · Inhibits osteoclast differentiation and activity · Decreases bone resorption · No residual effect
	PTH	Teriparatide	· The first anabolic gent · Exhibits a superior increase in bone mass compared to anti-resorptive agents · Increases the number of osteoblasts
	Romosozumab	Romosozumab	· Sclerostin monoclonal antibody · Inhibition of the Wnt signaling pathway · Dual action by promoting bone formation and temporarily inhibiting bone resorption

ARD: Anti-resorptive drug, PTH: Parathyroid Hormone, RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand.

4.3.2. Bisphosphonates

Bisphosphonates are the most well-known of the medications used for osteoporosis treatment. It is highly effective and is widely prescribed and used in South Korea. It is a potent ARD used to prevent pathological fractures caused by osteoporosis, osteomalacia, and malignancy-induced bone metastasis.[25,26] It is also used in the management of metabolic bone diseases, such as Paget's disease and osteogenesis imperfecta. The chemical structure of this medication, with a P-C-P backbone, is chemically stable. The two side chains, R1 and R2, that bind to two phosphate groups and a carbon atom increase its affinity for bones. In other words, bisphosphonates strongly bind to the bone mineral surface and are absorbed by osteoclasts during bone resorption. Eventually, the osteoclasts undergo apoptosis, and bone resorption is inhibited. Bisphosphonates can be administered orally or intravenously, and they are known to have a very long residual effect even after discontinuation. This property helps maintain the anti-resorptive effect, reducing the risk of side effects, and preserving the fracture-preventing effects.[27] However, the most significant side effect of bisphosphonates is medication-related osteonecrosis of the jaw (MRONJ). MRONJ occurs in patients with a history of bisphosphonate use. The symptoms include fever, fatigue, esophageal ulceration, anemia, edema, and osteonecrosis of the jaw. Concerns about the long-term safety

of bisphosphonates persist. MROMJ was first reported as a medication-related side effect by Marx[28] in 2003, primarily associated with intravenous bisphosphonate therapy among cancer patients, and many cases have been reported since then. To date, osteonecrosis of the jaw (ONJ), affecting mainly the maxilla and/or mandible is also common. Cases involving other bones have not been reported. This is thought to be primarily due to the relatively rapid bone turnover rate in the jawbone compared to other bones of the body. To diagnose MRONJ, a patient must have a history of using ARDs or anti-angiogenic agents, exposed bone or non-healing area of bone in the oral or extraoral region, persisting for more than 8 weeks, and no history of radiation therapy to the jawbone.

4.3.3. Denosumab

Denosumab is a potent ARD that inhibits RANKL, a human monoclonal antibody. Its mechanism of action involves specific binding to RANKL, thereby inhibiting the RANKL-RANK interaction, which disrupts functions such as the formation, differentiation, and activation of osteoclasts.[29] In a Phase III study (the "Fracture Reduction Evaluation of Denosumab in Osteoporosis Every Six Months" (FREEDOM) study), denosumab was proven to be a potent ARD, that increased bone mineral density in the spine, non-spine areas, and hip, and reduced fracture risks.[30] Subsequent extension studies have shown that the effects of denosumab can last for up to ten years.[29,31] However, when denosumab treatment is discontinued, bone turnover markers rise, and bone mineral density can decrease sharply within 12 months, increasing the risk of vertebral fractures to the levels seen before treatment. This phenomenon is referred to as the "rebound effect" and occurs due to a rapid increase in osteoclastic activity.[32] In other words, denosumab does not have a residual effect. Therefore, it is recommended to continue treatment without breaks. If denosumab is used and then discontinued, it is advisable to switch to a bisphosphonate or another ARD to maintain bone health. Denosumab, like other ARDs, has been associated with MRONJ. The use of ARDs is considered a risk factor for MRONJ. As such, the administration of bone resorption inhibitors can

lead to osteonecrosis, a condition where bone tissue is destroyed and loses its function due to inflammation, tumors, trauma, and other causes. Therefore, patients undergoing treatment with bone resorption inhibitors should be aware of the potential risk of ONJ.

4.3.4. Parathyroid hormone

Parathyroid hormone (PTH) was the first anabolic agent developed for osteoporosis treatment. It plays a crucial role in regulating calcium and phosphate metabolism and is primarily secreted by the parathyroid glands. In South Korea, teriparatide, a PTH analog, is available for osteoporosis treatment. Teriparatide is a biologically engineered product created by reassembling genes of human PTH from positions 1 to 34. Teriparatide is known for its excellent bone healing effects and superior bone formation compared to ARDs. It is often used in severe osteoporosis (e.g., patients with a bone mineral density of -5 to -6, those who have experienced vertebral fractures, or in cases of repeated fractures despite ARD treatment. When discontinuing teriparatide, bone mass can decrease to its pre-treatment state, so if discontinued, the use of another anabolic agent or ARD is recommended. However, teriparatide is relatively expensive and requires daily subcutaneous injections. It is also inconvenient as teriparatide must be stored refrigerated. Teriparatide has also been associated with osteosarcoma in animal experiments, depending on the dose and duration of treatment. Therefore, its use should be limited to approved indications. Considering the potential risk of malignancy, teriparatide should not be administered long-term, and it is contraindicated in patients with a family history of osteosarcoma and patients with cancer or cancer history. To date, there is no clear

association between teriparatide and MRONJ. On the contrary, daily low-dose teriparatide injection can be used to treat MRONJ as well as osteoporosis.[33]

4.3.5. Romosozumab

Romosozumab is a monoclonal antibody that targets sclerostin, a protein involved in bone recovery and skeletal development through the Wnt signaling pathway. Sclerostin is produced by osteocytes and plays a crucial role in inhibiting bone formation and reducing osteoblastic activities. Romosozumab binds to sclerostin to inhibit its action, which in turn, increases bone matrix production while regulating the expression of key factors, such as RANKL and OPG, leading to suppressed bone resorption. Its dual action is a distinctive feature. Romosozumab promotes bone formation in the early stages and partially inhibits bone resorption in the later stages of treatment. Romosozumab received approval from the U.S. Food and Drug Administration (FDA) in April 2019 and was introduced in South Korea in May 2019, raising expectations regarding its effectiveness. However, there have been reported cases of MRONJ associated with romosozumab.[34,35] Current data suggest that patients treated with romosozumab have a similar risk of MRONJ as those treated with bisphosphonates.

4.4 Orthodontic treatment with bisphosphonates

In animal OTM experiments, various effects have been observed when applying specific drugs. In this study, we investigated how different medications used for osteoporosis drugs impact orthodontic treatment.

Bisphosphonates enter osteoclasts through endocytic vacuoles, where they deactivate cellular functions and reduce cell lifespan.[36,37] Therefore, long-term administration of bisphosphonates can slow down the rate of OTM by inhibiting bone resorption, delaying the remodeling process, and potentially extending the duration of orthodontic treatment.[38]

4.4.1. Orthodontic treatment with bisphosphonates in ovariectomized rats

Table 2 is a list of published animal experiments involving the use of bisphosphonates and OTM in an osteoporosis model. All experiments induced osteoporosis by performing ovariectomy in female rats and applying a nickel-titanium (Ni-Ti) coil spring between the maxillary first molar and incisor to move the first molar mesially. In 2012, Sirisoontorn et al.[21] and in 2013, Hashimoto et al.[39] initiated intraperitoneal zoledronate (ZOL) administration two weeks after ovariectomy and measured OTM using micro-computed tomography (micro-CT). In 2015, Salazar et al.[40] started subcutaneous alendronate (ALN) injections 90 days

after ovariectomy and measured OTM histologically In 2019, Dongle et al.[22] began intraperitoneal risedronate (RIS) administration two weeks after ovariectomy and measured tooth movement directly. In all these studies, bisphosphonates were injected into animal models with induced osteoporosis, which led to inhibited tooth movements.

Table 2. Characteristics of orthodontic tooth movement animal experiments with ovariectomy and bisphosphonate administration.

	Study (or article)	Subjects [species; gender; age; weight]	Medication [substance; dosage; injection; administration]	Tooth movement model	Group characteristics [no]	Type of anch-orage	Measurement methodology	Outcome	Results	Side effects	Journal
1	Dongle Wu et al. 2019	SD rats Female 10wks 200-220g	BP (RIS) 10 μg/kg Every three days Administered intraperitoneally Two weeks after OVX	NiTi coil spring placed between the maxillary left M1 and incisor **Force application**: 3, 7 and 14 days **Force**: nm	OVX (n=15) OVX+risedronate (n=15) Sham (n=15) Total (n=45)	Incisor	**Direct measurements**: With digital caliper; Measure the distance between the mesial wall of the maxillary left M2 and the distal wall of the maxillar left M1	Distance of tooth movement; TRAP staining; IHC staining (RANKL, OPG, and cathepsin K expressions)	RIS decreased the OTM in OVX rats	-	Archives of Oral Biology (AOB)
2	Salazar et al. 2015	Wistar rats Female 8wks	BP (ALN) 1 or 2 mg/kg Twice a wk Subcutaneous injection For 90 days following OVX	NiTi coil spring placed between the maxillary right M1 and incisor **Force application**: 5, and 7 days **Force**: 50cN	OVX (n=12) OVX+ALN1 (n=12) OVX+ALN2 (n=12) Sham (n=12) Total (n=48)	Incisor	**Histological cuts**: The smallest distance between the distal face of the M1 and mesial face of the M2	Distance of tooth movement; H&E staining (quantification of interradicular alveolar bone)	ALN reduced OTM in OVX rats	-	Archives of Oral Biology (AOB)
3	Hashimoto et al. 2013	Wistar rats Female 10wks 170-180g	BP (ZOL) 3.2 μg/kg Once every 2 wks Peritoneal cavity Two weeks after OVX	NiTi coil spring placed between the maxillary left M1 and the central incisor **Force application**: 2 wks **Force**: 25g	OVX (n=7) OVX+ZOL (n=7) Sham (n=7) Total (n=21)	Incisor	**Micro-CT images:** The distance from the most distal contact point of the maxillary left M1 and the most mesial contact point of te maxillary M2; In vivo three-dimensional (3D) analysis	Distance of tooth movement; Micro-CT (tibia analysis)	ZOL suppressed OTM in OVX rats	-	The Angle Orthodontist (AO)
4	Sirisoontorn et al. 2012	Wistar rats Female 10wks 170-180g	BP (ZOL) 1.6 μg/kg Once a wk (for a total of 6 times) Peritoneal cavity Two weeks after OVX	NiTi coil spring placed between the maxillary left M1 and the central incisor **Force application**: 4 wks **Force**: 25g Four weeks after OVX or sham	OVX (n=5) OVX+ZOL (n=5) Sham (n=5) Total (n=15)	Incisor	**Micro-CT images**: The distance from the most distal contact point of the maxillary left M1 and the most mesial contact point of the maxillary M2; In vivo three-dimensional (3D) analysis	Distance of tooth movement; SEM (root resorption); Micro-CT (areas of the resorption craters)	ZOL suppressed OTM in OVX rats	-	American Journal of Orthodontics and Dentofacial Orthopedics (AJODO)

SD: Sprague-Dawley, RIS: Risedronate, ALN: Alendronate, ZOL: Zoledronate, CLO: Clodronate, Ni-Ti: Nickel Titanium, M1: First molar, M2: Second molar, 3D: Three-dimensional, RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand, OPG: Osteoprotegerin, H&E: Hematoxylin and Eosin, TRAP: Tartrate-Resistant Acid Phosphatase, IHC: Immunohistochemistry, SEM: Scanning electron microscopy, OVX: Ovariectomy.

4.4.2. Alendronate

Table 3 is a list of publications that performed experiments involving ALN administration in male rats followed by OTM. In most studies,[41-44] a coil spring was applied between the maxillary first molar and incisor to move the maxillary first molar mesially. However, in the 2022 study by Moradinejad et al.[45] OTM was accomplished by using a wire to create separation between the incisors. Despite different drug administration strategies, the results consistently showed a decrease in OTM when ALN was administered, both before and during OTM. Over the years, many researchers have chosen to use male animals for experiments to avoid the potential influences from the estrous cycle or physiological changes in female animals. However, similar results were obtained in experiments involving different genders (compare Tables 2 and 3).

Table 3. Characteristics of orthodontic tooth movement animal experiments with alendronate among bisphosphonates.

	Study (or article)	Subjects [species; gender; age; weight]	Medication [substance; dosage; injection; administration	Tooth movement model	Group characteristics [no]	Type of anch-orage	Measurement methodology	Outcome	Results	Side effects	Journal
1	Moradine-jad et al. 2022	Wistar rats Male 12wks 252.5 ± 18.9g	BP (Sodium alendronate; ALN) 7 mg/kg Once a week for a total of 5 times	stainless steel (SS) wire with 3 helixes was placed on the maxillary incisors **Force application**: 2 wks **Force**: 30 ± 0.1g	Vitamin D3 (n=8) ALN + D3 (n=8) ALN (n=8) Control (n=8) Total (n=32)	-	**Direct measurements**: With digital caliper; The distal-to-diatal distance between the central teeth	Distance of tooth movement; H&E staining (capillaries, lacunae, osteoclasts, osteoblasts)	ALN reduces OTM	-	American Journal of Orthodontics and Dentofacial Orthopedics (AJODO)
2	Lopes et al. 2021	Wistar rats Male 10-12wks 180g	BP (ALN) 1 or 6 mg/kg 5 wks Oral	NiTi coil spring placed between the upper left M1 and central incisors **Force application**: 2 wks **Force**: 50g	ALN1 (n=10) ALN6 (n=10) Control (n=10) Total (n=30)	Incisor	**Micro-CT images**: The greatest projection of the distal face of the M1 to the point with the greatest projection of the mesial face of the M2	Distance of tooth movement; Micro-CT (BV, Tb.Th, Tb.N and Tb.Sp)	ALN reduced tooth displacement	-	Orthodontics & Craniofacial Research (OCR)
3	Franzoni et al. 2017	Wistar rats Male 10 month 500g	BP (ALN) 2.5 mg/kg BP (ZOL) 0.1 mg/kg Once a day (25 days before OTM started and during the experimental period of 10 days) SC	SS coil spring placed between the maxillary left M1 and the upper incisors **Force application**: 10 days **Force**: 0.4N	ALN (n=5) ZOL (n=5) Control (n=5) Total (n=15)	Incisor	**Direct measurements**: With digital caliper; The distance between the distal side of the M3 and the mesial side of the M1	Distance of tooth movement; Toluidine blue staining (the number of blood vessels, fibroblasts cells and osteoclasts); Dominici staining (inflammatory cells);	ZOL delay OTM		Orthodontics & Craniofacial Research (OCR)

SD: Sprague-Dawley, ALN: Alendronate, ZOL: Zoledronate, SC: subcutaneous, SS: stainless steel, Ni-Ti: Nickel Titanium, M1: First molar, M2: Second molar, H&E: Hematoxylin and Eosin, BV/TV: Bone volume and total volume, Tb.Th : Trabecular thickness, Tb.N : Number of trabeculae, Tb.Sp: Separation between trabeculae.

Table 3. Continued

	Study (or article)	Subjects [species; gender; age; weight]	Medication [substance; dosage; injection; administration]	Tooth movement model	Group characteristics [no]	Type of anch-orage	Measurement methodology	Outcome	Results	Side effects	Journal
4	Kaipatur et al. 2013	SD rats Female 12 wks	BP (ALN) 0.015 mg/kg 8 or 12 wks (twice weekly) SC (during OTM, or before and after OTM)	NiTi coil spring placed between the maxillary right M1 and microimplant **Force application**: 8 wks **Force**: 50g	BP1 (n=5) BP2 (n=5) Control1 (n=5) Control2 (n=5) Total (n=20)	Implant	**Micro-CT**: The linear distance from the most convex contact area between the maxillary right first and second molars	Distance of tooth movement; H&E staining (bone resorption)	Pre-dosed ALN drug use will inhibit tooth movement 4 and 8 weeks of tooth movement	-	American Journal of Orthodontics and Dentofacial Orthopedics (AJODO)
5	Karras et al. 2009	SD rats	BP (ALN) 7 mg/kg per week (5 wks) gavage technique	NiTi coil spring placed between the maxillary M1 and central incisors **Force application**: 2 and 4 wks **Force**: 50g	ALN (n=11) Control (n=11)	Incisor	**Plaster models**: digital measurement using software; the diastema distal to the maxillary first molar => the distance between the M1 and M2	Distance of tooth movement;	ALN reduced tooth displacement;	-	American Journal of Orthodontics and Dentofacial Orthopedics (AJODO)

SD: Sprague-Dawley, ALN: Alendronate, ZOL: Zoledronate, SC: subcutaneous, SS: stainless steel, Ni-Ti: Nickel Titanium, M1: First molar, M2: Second molar, H&E: Hematoxylin and Eosin, BV/TV: Bone volume and total volume, Tb.Th: Trabecular thickness, Tb.N: Number of trabeculae, Tb.Sp: Separation between trabeculae.

4.4.3. Zoledronate

Table 4 is a list of published experiments involving ZOL administration in male rats followed by OTM. In most studies[46-48] the maxillary first molar was moved mesially with a Ni-Ti coil spring, and the incisor served as an anchor system. However, in the 2016 study by Gonzalez et al.[49] a mini-screw was placed at the root portion of the incisor as an anchor system. The methods of drug administration varied between studies including intravenous injection, subcutaneous injection, and local injection. Most studies reported that ZOL reduced OTM, except for the 2016 study by Brunet et al.[47] which reported no effects.

Table 4. Characteristics of orthodontic tooth movement animal experiments with zoledronate among bisphosphonates.

	Study (or article)	Subjects [species; gender; age; weight]	Medication [substance; dosage; injection; administration]	Tooth movement model	Group characteristics [no]	Type of anch-orage	Measurement methodology	Outcome	Results	Side effects	Journal
1	De Sousa et al. 2021	Wistar rats Male 10wks 200-250g	BP (ZOL) 0.2 or 1.0 mg/kg Days 0, 7, 14 and 42 IV (dorsal penile vein)	NiTi coil spring placed between the maxillary left M1 and the maxillary left incisor **Force application**: 21 day **Force**: 50g	ZOL 0.2 (n=6) ZOL 1.0 (n=6) Saline (n=6) Naive (n=6) Total (n=24)	Incisor	**Direct measurements**: With digital caliper; The distance between the upper left M1 and the upper central incisor; The most cervical point	Distance of tooth movement; H&E staining (osteocytes, osteoclasts and alveolar bone resorption); Mallory (blood vessels and collagen fibers); Picrosirius red staining (collagen fibers); Mivro-CT; SEM (bone resorption)	ZOL reduced tooth displacement	-	Scientific Reports (SR)
2	Gonzalez et al. 2016	SD rats Male 420-460g	BP (ZOL) 16 μg single dose locally (the palatal mocusa)	NiTi coil spring placed between the maxillary right M1 and the mini-screw **Force application**: 21 days **Force**: 50g	ZOL (n=12) OPG-Fc (n=12) Control (n=12) Total (n=36)	Mini-screw	**Plaster models**: digital measurement using software;	Distance of tooth movement; H&E staining; Trichromic staining; IHC staining (ASIC2, Col1, MMP-2, MMP-9, RANK, Runx2, S100P, TRPV4 and Vimentin Micro-CT (bone volume fraction (BVF))	ZOL reduced tooth displacement	-	Orthodontics & Craniofacial Research (OCR)
3	Brunet et al. 2016	Wistar rats Male 9 wks 300-350g	BP (ZOL) 0.1 mg/kg single dose (one week prior to start of tooth movement) IP	NiTi coil spring placed between the maxillary right M1 and the maxillary right incisor **Force application**: 3, 7 and 14 days **Force**: 30cN	ZOL+OTM (n=30) ZOL+nOTM (n=30) nZOL+OTM (n=30) nZOL+nOTM (n=30) Total (n=120)	Incisor	**Direct measurements**: With digital caliper; The distance between the right upper M1 and the upper central incisor	Distance of tooth movement; H&E (root resorption); TRAP (osteoclasts); Picrosirius red staining (the expression of mature and immature collagen)	ZOL did not reduce tooth displacement		Brazilian Dental Journal (BDJ)
4	Ortega et al. 2012	SD rats Male	BP (ZOL) 16 μg single dose locally	NiTi coil spring placed between the maxillary left M2 and central incisors **Force application**: 21 days **Force**: 10g	ZOL (n=15) Control (n=15) Total (n=30)	Incisor	**Micro-CT and direct measurements**: With cephalograms; The radiographic parameters.; The distance between the contact points of the maxillary left M2 and M3	Distance of tooth movement; Micro-CT; H&E staining	A single, small, locally applied dose of ZOL was sufficient to provide maximum anchorage in extraction space closure	-	American Journal of Orthodontics and Dentofacial Orthopedics (AJODO)

SD: Sprague-Dawley, IV: Intravenous, IP: Intraperitoneal, OTM: orthodontic tooth movement, ZOL: Zoledronate, Ni-Ti: Nickel Titanium, M1: First molar, M2: Second molar, M3: Third molar, 3D: Three-dimensional, RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand, OPG: Osteoprotegerin, H&E: Hematoxylin and Eosin, BV/TV: Bone volume and total volume, Tb.Th : Trabecular thickness, Tb.N : Number of trabeculae, Tb.Sp: Separation between trabeculae, SEM: Scanning electron microscopy, IHC: Immunohistochemistry, BVP: bone volume fraction, TRAP: Tartrate-Resistant Acid Phosphatase.

4.4.4. Risedronate

Table 5 lists the published experiments involving RIS administration in male rats followed by OTM. Both of these studies were conducted in the 1990s and involved moving the maxillary first molar in the buccal direction. Adachi et al.[50] suggested that local administration of RIS could reduce tooth movement and prevent relapse. Subsequently, in 2019, Dongle et al.[22] demonstrated the effectiveness of RIS in rats with induced osteoporosis (Table 2).

Table 5. Characteristics of orthodontic tooth movement animal experiments with risedronate among bisphosphonates.

	Study (or article)	Subjects [species; gender; age; weight]	Medication [substance; dosage; injection; administration]	Tooth movement model	Group characteristics [no]	Type of anch-orage	Measurement methodology	Outcome	Results	Side effects	Journal
1	Igar et al. 1996	Wistar rats Male 9-10 wks	BP (RIS) 125, 250 or 500 μmol/L every 3 days administered locally (sub-periosteum area)	The SE spring placed between the maxillary right and left M1 teeth moved in a buccal direction **Force application**: 3 wks **Force**: 165 mN	RIS Control Total (n=53)	-	-	H&E staining (odontoclasts and root resorptive area)	Root resorption could be prevented even by the topical administration of the RIS	-	Journal of Dental Research (JDR)
2	Adachi et al. 1994	Wistar rats Male 9-10 wks 227g	BP (RIS) 125, 250 or 500 μmol/L every 3 days (3 wks, the spring was removed) administered locally (sub-periosteum area)	The SE (NiTi) spring placed between the maxillary right and left M1 teeth moved in a buccal direction **Force application**: 3 wks **Force**: 165mN	RIS Control Total (n=126)	-	**Plaster models**: sliding calipers; The distance between the crests of the mesiopalatal cusps of the first molars	Distance of tooth movement	The local administration of RIS reduced tooth movement	-	Journal of Dental Research (JDR)

RIS: Risedronate, SE: Standardized Expansion, Ni-Ti: Nickel Titanium, M1: First molar, H&E: Hematoxylin and Eosin.

4.4.5. Clodronate and pamidronate

Table 6 lists the published animal experiments involving OTM and the administration of clodronate (CLO) or pamidronate (PAM). These experiments were conducted not only in rats but also in rabbits.[51] Of note, both male and female animals were used, unlike the studies mentioned earlier (Tables 3 to 5).[52,53] In 2014, Venkataramana et al.[51] and in 2010, Choi et al.[53] used a Ni-Ti coil spring placed between the maxillary first molar and incisor to move the maxillary first molar mesially. Both studies measured OTM directly. In 2004, Lin et al.[54] moved the teeth buccally and measured the amount of OTM using plaster models. In 2017, only Nakas et al.[52] inserted an elastic band between the incisors to induce movement and measured tooth displacement using the plaster models. Various methods of drug administration were employed in these studies, and all of them demonstrated reduced OTM.

Table 6. Characteristics of orthodontic tooth movement animal experiments with clodronate and pamidronate among bisphosphonates.

	Study (or article)	Subjects [species; gender; age; weight]	Medication [substance; dosage; injection; administration]	Tooth movement model	Group characteristics [no]	Type of anch-orage	Measurement methodology	Outcome	Results	Side effects	Journal
1	Nakas et al. 2017	Wistar rats Male and Female 8-9 wks 343g	BP (CLO) 10 or 2.5 mMol 3 or 7 day intervals (6 or 2 times) locally (subperiosteal area)	Insertion of the elastic band between the incisors **Force application**: 3 wks **Force**: nm	CLO1 (n=15) CLO2 (n=15) CLO3 (n=15) CLO4 (n=15) Total (n=60)	-	**Plaster models**: digital measurement using software; The distance between incisores and molars in the trated and control side **method error assessment**: Yes	Distance of tooth movement; H&E staining; IHC staining (calcitonin expression)	CLO can reduce tooth movement	-	Bosnian Journal of Basic Medical Sciences (BJBMS)
2	Venkatar-amana et al. 2014	New Zealand white rabbits Male 16 wks 3.75 kgs	BP (PAM) 1.5 mg/kg 1, 7 and 14 days IP	NiTi coil spring placed between the lower M1 and the incisors **Force application**: 3 wks **Force**: 100g	BP (n=10) Control (n=10) Total (n=20)	incisor	**Direct measurements**: standard metric scales; The distance between the mesioocclusal margin of the second molar to the disto-occlusal margin of the first molar.	Distance of tooth movement; H&E staining (osteoclasts)	PAM reduced tooth displacement	-	Journal of International Oral Health (JIOH)
3	Choi et al. 2010	Wistar rats sex-matched 8 wks 180-230g	BP (CLO) 2.5 or 10 mmol/L every third day locally (subperiosteum adjacent)	NiTi coil spring placed between the maxillary M1 and incisors **Force application**: 3, 6, 9, 12 and 15 days **Force**: 60g	CLO1 (n=18) CLO2 (n=18) Control (n=18) Total (n=54)	Incisor	**Direct measurements**: With digital caliper; The distance between the tip of the maxillary central incisors and the mesiobuccal cusp tip of the maxillary first molars	Distance of tooth movement; H&E staining (osteoclast and root resorption); Fluorescence microscopy images (bone labels)	CLO reduced tooth displacement	-	American Journal of Orthodontics and Dentofacial Orthopedics (AJODO)
4	Lin et al. 2004	Wistar rats Male 7 wks 180g	BP (CLO) 2.5, 10 or 40 mM every third day locally (sub-periosteum area)	The SE spring placed between the maxillary M1 teeth moved in a buccal direction **Force application**: 3	ALN1 ALN2 ALN3 Total (n=26)	-	**Plaster models**: sliding callipers; The distance between the crests of the mesio-palatal cusps of the M1	Distance of tooth movement; TRAP staining (osteoclasts and bone resorption)	The local administrati on of CLO caused reduction in tooth movement		The European Journal of Orthodontics (EJO)

IP: Intraperitoneal, CLO: Clodronate, PAM: Pamidronate, Ni-Ti: Nickel Titanium, M1: First molar, H&E: Hematoxylin and Eosin, IHC: Immunohistochemistry, TRAP: Tartrate-Resistant Acid Phosphatase.

Most experiments involved rats, while some used mice or rabbits. Clinically, cases of failed orthodontic treatment due to the inability of teeth to move as expected following drug administration have been reported.[27] In situations where OTM is challenging, studies have suggested the administration of dihydroxycholecalciferol (Vitamin D3) supplementation to enhance osteoblastic differentiation, increase the number of osteoclasts and osteoblasts, and regulate calcium homeostasis. The addition of Vitamin D3 did not diminish the effects of the medication.[45] As bisphosphonates are commonly used for the treatment of osteoporosis or bone loss, some orthodontic patients may also be using these medications. A previous case report described patients, who were taking alendronate regularly, having successful orthodontic treatment by taking drug holidays.[55] However, bisphosphonates are associated with the risk of MRONJ. Therefore, if a patient is on bisphosphonates, close communication between the orthodontist and the primary care physician is advisable. This is especially important for patients with a history of high-dose intravenous bisphosphonate administration, cancer, or oral surgeries including extractions.

4.5. Orthodontic treatment with denosumab

Denosumab-related animal studies are presented in Table 7. For denosumab, only a limited number of animal experiments were found, with the earliest published in 2017 and the most recent in 2022.[56,57] The scarcity of results can be attributed to the difficulty in obtaining RANKL antibodies for mice. Notable differences compared to other studies include the exclusive use of mice as experimental subjects and the utilization of anti-mouse RANKL antibodies. In 2017, Ayumi et al.[56] locally administered the drug to the target site during the OTM period in 12-week-old male mice. In 2022, Yoshimatsu et al.[57] injected the drug via a single intraperitoneal injection 21 days before the initiation of OTM in 7-8-week-old male mice. The selected age of the mice corresponds to young adulthood, but in reality, osteoporosis is more prevalent among middle-aged and elderly individuals. Moreover, osteoporosis is more common in women, although it also affects men. Furthermore, while osteoporosis drugs were used, the experiments were not conducted in osteoporosis models. These study limitations need to be addressed in the future. It remains uncertain whether similar or different results would be observed if the same drugs were administered to different individuals e.g., the young adults and the elderly. Nevertheless, both studies reported that anti-mouse RANKL antibodies reduced OTM.

Table 7. Characteristics of orthodontic tooth movement animal experiments including denosumab.

	Study (or article)	Subjects [species; gender; age; weight]	Medication [substance; dosage; injection; administration]	Tooth movement model	Group characteristics [no]	Type of anch-orage	Measurement methodology	Outcome	Results	Side effects	Journal
1	Yoshimat-su et al. 2022	WT (C57BL6/J) mice Male 7-8 wks	Anti-mRANKL antibody 100 μg Once (21 days before OTM started) IP	NiTi coil spring between the maxillary left M1 and the upper incisor **Force application**: 10 days **Force**: 10g	Ab nAb	Incisor	**Stereoscopic microscope**: The shortest distance between the M1 and M2	Distance of tooth movement; TRAP staining (TRAP-positive cells, root resorption)	The anti-mRANKL antibody reduced the distance of tooth movement	-	Journal of Dental Sciences (JDS)
2	Ayumi et al. 2017	WT mice Male 12 wks	Anti-mouse RANKL monoclonal antibody 10 μg Every 8 days (during OTM) locally (buccal and palata gingiva)	NiTi coil spring between the maxillary left M1 and the maxillary incisors **Force application**: 8 days **Force**: nm	Ab (n=3-4) Control (n=3-4)	Incisor	**Micro-CT**: measuring the micro-CT images; The closest distance between the M1 and the M2	Distance of tooth movement	The anti-mRANKL antibody reduced the distance of tooth movement		Scientific Reports (SR)

WT: Wild-type, OTM: Orthodontic tooth movement, IP: Intraperitoneal, M1: First molar, M2: Second molar, ab: Antibody, Ni-Ti: Nickel Titanium, TRAP: Tartrate-Resistant Acid Phosphatase.

Bisphosphonates and denosumab are ARDs that inhibit osteoclast differentiation and function, resulting in decreased bone resorption and bone remodeling.[58] The key difference between these two drugs is that bisphosphonates maintain their effect for a certain period even after discontinuation, while the effect of denosumab largely disappears within six months, with no residual effect. Additionally, there are two main types of denosumab. Prolia, used in osteoporosis treatment, is administered at a low dose of 60 mg via subcutaneous injection every six months, while Xgeva, used in solid cancers with bone metastasis or hypercalcemia, is administered at a high dose of 120 mg every four weeks. Denosumab has a reported half-life of about 25 days.[59] During this half-life, the effects gradually diminish to the original state. The duration of this decline varies from weeks to months depending on the dosage. Therefore, determining the timing of dental treatment based on the dosing regimen is crucial. Patients receiving Prolia every six months typically undergo tooth extraction around 3 to 5 months after denosumab administration, followed by healing and re-administration of the drug in the sixth month. Discontinuing denosumab can lead to rapid deterioration in some patients including vertebral fractures due to a rebound effect.[60] Furthermore, poorly timed treatment increases the likelihood of MRONJ. Hence, the timing of dental treatment can significantly impact the outcome. However, animal experiments investigating the simultaneous administration of RANKL antibodies and OTM, or those focusing

solely on short-term effects, have not provided sufficient evidence to support an optimal timeframe for OTM. There have been cases where patients receiving denosumab were diagnosed with MRONJ just one year after starting orthodontic treatment, suggesting that OTM could be a contributing factor.[61] Importantly, patients who are on high doses of denosumab should keep in mind the risk of MRONJ, similar to bisphosphonates.

4.6. Orthodontic treatment with PTH

Table 8 provides a list of animal experimental studies on OTM with PTH application. In all the studies, the first maxillary molar was moved distally using coil springs. However, in the study by Soma et al.[62] in addition to coil springs, an elastic band was inserted between the maxillary first and second molars to facilitate OTM. Only Salazar et al. (2011)[63] induced osteoporosis by performing ovariectomy in female rats and evaluated the effect of PTH on OTM. They reported that compared to the OVX group, the group receiving PTH injections in the osteoporosis model showed increased tooth movement, suggesting a potential for shortening the duration of orthodontic treatment. Until 2013, only tooth movement and histological analysis were conducted, but in 2021, Lu et al.[64] utilized micro-CT analysis in their study. In these studies, PTH was injected once at the target site but in various ways. All available studies suggest that PTH accelerates or increases OTM. PTH is known to stimulate both osteoblasts and osteoclasts, thereby promoting bone turnover and normalizing bone density. Furthermore, PTH may directly impact osteoblasts during orthodontic treatment.[62]

Table 8. Characteristics of orthodontic tooth movement animal experiments including PTH.

	Study (or article)	Subjects [species; gender; age; weight]	Medication [substance; dosage; injection; administration]	Tooth movement model	Group characteristics [no]	Type of anch-orage	Measurement methodology	Outcome	Results	Side effects	Journal
1	Lu et al. 2021	Wistar rats Male 7 wks 200-220g	PTH 0.1 ml Once (with PECE hydrogel) locally	NiTi coil springs were ligated bilaterally between the maxillary M1 and the incisors **Force application**: 2 wks **Force**: 40g	PTH PTHrP Total (n=40)	Incisor	**Micro-CT**: Digital slide calipers on micro-CT images; The distance between the M1 and the M2 at the cervical level	Distance of tooth movement; Micro-CT; H&E staining, TRAP staining (TRAP-positive cells); IHC staining (RANKL, OPG)	The local injection of PTH increased OTM distance	-	Journal of Periodontal Research (JPR)
2	Li et al. 2013	Wistar rats Male 8 wks 200 ± 10 g	PTH (1-34) 40 μg/kg Daily Subcutaneous injection	Orthodontic elastic coil spring between the maxilary right M1 and the incisors **Force application**: 0, 3, 6, 9 and 12 days **Force**: 40g	PTH (n=30) Control (n=30) Total (n=60)	Incisor	**Direct measurements**: With vernier caliper; Measure the distance between the Maxillary M1 and M2	Distance of tooth movement; H&E (Multinucleated clastic cells); TRAP staining; IHC staining (RANKL, OPG and IGF-1)	The acceleration effect of PTH on tooth movement has been shown	-	American Journal of Orthodontics and Dentofacial Orthopedics (AJODO)
3	Salazar et al. 2011	Wistar rats Female 8 wks	PTH (TPTD) 30 μg/kg Daily Subcucaneous injection 90 days after OVX	NiTi coil spring between the maxillary left M1 and the incisors **Force application**: 5 and 7 days **Force**: 40cN	OVX (n=16) OVX+TPTD (n=16) Sham (n=16) Total (n=48)	Incisor	**Histological cuts**: The distance between the M1 and M2	Distance of tooth movement; H&E staining (thickness of the PDL and osteoclasts)	The rate of OTM was greater in OVX and OVX+TPTD when compared with the control	-	American Journal of Orthodontics and Dentofacial Orthopedics (AJODO)
4	Soma et al. 2000	Wistar rats Male 20 wks 350-380g	PTH 0.1 or 1.0 μg/μL every other day Locally (subperiosteum) or subcucaneous injection	Orthodontic coil spring between the maxillary M1 and the incisors **Force application**: 12 days **Force**: 30g	Local Veh in MC (n=8) Local PTH0.1 in MC (n=8) Local PTH1 in MC (n=8) Local PTH1 in saline (n=8) Systemic PTH1 (n=8) Local PTH1 nOTM (n=8) Control (n=8) Total (n=56)	Incisor	**Plaster models**: Microscope with calipers; The interproximal distance between M1 and M2	Distance of tooth movement; H&E staining (bone resorption)	The local injection of PTH increased OTM distance	-	Journal of Dental Research (JDR)

PTH: Parathyroid Hormone, TPTD: Teriparatide, EXP: Experiment, MC: Methylcellulose, H&E: Hematoxylin and Eosin, TRAP: Tartrate-Resistant Acid Phosphatase, IHC: Immunohistochemistry, RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand, OPG: Osteoprotegerin, IGR-1: Insulin-like Growth Factor 1, BV/TV: Bone volume and total volume, BS/BV: Bone surface/bone volume, Tb.Th: Trabecular thickness, Tb.N: Number of trabeculae, Tb.Sp: Separation between trabeculae, PDL: periodontal ligament.

Table 8. Continued

	Study (or article)	Subjects [species; gender; age; weight]	Medication [substance; dosage; injection; administration]	Tooth movement model	Group characteristics [no]	Type of anch-orage	Measurement methodology	Outcome	Results	Side effects	Journal
5	Soma et al. 1999	Wistar rats Male 350-400 g 220g	PTH (1-84) 1, 3 and 10 μg/100g of body weight continuous infusion subcucaneous infusion	Elastic band was inserted between the upper M1 and M2 **Force application**: 3 days **Force**: No	EXP 1. Dose PTH1 (n=6) PTH3 (n=6) PTH10 (n=6) Control (Veh) (n=6) Total (n=24)	-	**Direct measurements**: contact gauges; The interproximal distance between M1 and M2	Tooth serperation; H&E staining (osteoclasts and bone resorption)	systemic infusion of PTH accelerated OTM; PTH(1–34) at 4mg effect comparable to that of PTH(1–84) at 10g;	-	Journal of Bone and Mineral Research (JBMR)
			PTH (1-84) 10 μg/100g of body weight 48, 72, 120, and 168 h subcucaneous infusion	Elastic band was inserted between the right M1 and M2 **Force application**: 0, 24, 72 and 120 h **Force**: No	EXP 2. Time Group 1 (n=24) Group 2 (n=24) Total (n=48)	-	**Direct measurements**: contact gauges; The interproximal distance between M1 and M2	Tooth seperation; H&E staining (osteoclasts and bone resorption)			
			PTH (1-84 and 1-34) PTH (1-84) 10 μg/100g, PTH (1-34) 0.4 and 4 μg/100g, and PTH (1-34) 4 μg/100g continuous infusion or dailiy subcucaneous infusion or subcucaneous injection	Orthodontic coil spring between the upper M1 and the incisors **Force application**: 12 days **Force**: 30g	EXP 3. PTH(1-84) vs. PTH(1-34) PTH(1-84)10 infusion (n=8) PTH(1-34)0.4 infusion (n=8) PTH(1-34)4 infusion (n=8) PTH(1-34)4 injection (n=8) Veh (n=8) Total (n=40)	Incisor	**Plaster models**: Microscope with calipers; The interproximal distance between M1 and M2	Distance of tooth movement; H&E staining (osteoclasts and bone resorption)			

PTH: Parathyroid Hormone, TPTD: Teriparatide, EXP: Experiment, MC: Methylcellulose, H&E: Hematoxylin and Eosin, TRAP: Tartrate-Resistant Acid Phosphatase, IHC Immunohistochemistry, RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand, OPG: Osteoprotegerin, IGR-1: Insulin-like Growth Factor 1, BV/TV: Bone volume and total volume, BS/BV: Bone surface/bone volume, Tb.Th: Trabecular thickness, Tb.N:

4.7. Orthodontic treatment with romosozumab

Although research has suggested excellent effects with romosozumab, no animal experimental study specifically focused on orthodontic treatment using romosozumab has been reported. However, previous studies that applied orthodontic forces to sclerostin-injected rats have demonstrated accelerated tooth movement and increased osteoblast formation.[12,65] Thus, anti-sclerostin antibodies may initially increase tooth movement but gradually decrease it. As romosozumab is a recently introduced drug, further research is needed to evaluate its impact on orthodontic treatment and its association with MRONJ.

4.7.1. Administration of romosozumab in ovariectomized rat

Rodents have been widely used in biomedical research, OTM, and orthodontically induced root resorption studies due to their anatomical and physiological similarities. The ovariectomized rat model, which mimics the postmenopausal state in women, has been recognized as a model for postmenopausal osteoporosis because following ovariectromy, estrogen is not produced and the rats exhibit similar patterns of bone loss as humans.[6,21,22,66-68] In particular, when ovariectomy is performed in mature rat models aged around 3-6 months, it leads to premature menopause and bone loss, making it suitable for alveolar bone loss research.[67]

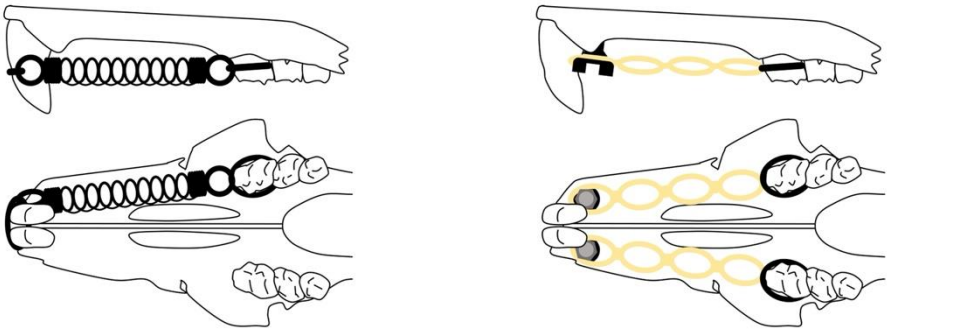
Previous studies have shown that ovariectomized rats experience faster OTM and a significant increase in osteoclast formation compared to non-ovariectomized rats.[22,68] In the present study investigated the effects of romosozumab on orthodontic treatment and the impact of the drug on the periodontium in an ovariectomized rat model with induced osteoporosis. To induce osteoporosis similar to that observed clinically, bilateral ovariectomy was performed on 12-week-old adult female rats to deplete estrogen and induce bone loss. Ovariectomy is known to trigger degenerative changes in bone after 2 weeks. Although alveolar bone turnover is slower than that in the tibia, previous studies have shown a significant reduction in bone mass within 2-3 months after ovarian removal.[69,70] The time frame applied to this study was based on previous findings.[8,71-73]

4.7.2. Orthodontic tooth movement

4.7.2.1. Coil spring vs. elastic band

Clinically, Ni-Ti coil springs and elastomeric power chains are most commonly used for delivering orthodontic forces to achieve space closure (Figure 16). Both materials have the tendency to return to their original state, thereby exerting forces on the teeth to be moved and the anchoring system and inducing experimental OTM.[74] Due to its shape memory and pseudoelasticity characteristics, Ni-Ti has been utilized for orthodontic space closure via Ni-Ti coil springs. Elastomeric

power chains are employed for leveling and aligning teeth, closing spaces, and maintaining orthodontic archwires in place. Power chains provide significant force initially but the force tends to decline over time.[75] While *in vitro* studies have indicated that elastomeric power chains experience a rapid loss in force compared to coil springs and are more influenced by the oral environment,[76,77] there have been limited studies comparing the efficiency of these two materials.[15,78] Moreover, a previous report has also suggested no significant difference in outcomes between the two methods,[79] Some studies have shown that the use of coil springs results in faster OTM but with an increased risk of tipping and loss of anchorage.[80] As evidenced in Tables 2 to 8, most of the existing literature has predominantly employed coil springs to induce OTM. Thus, when conducting long-term experiments with a reproducible experimental animal OTM model, Ni-Ti coil springs have been recommended. Elastomeric power chains are deemed suitable for short-term experiments, though it is worth noting that their force can be maintained with periodic replacements. The duration of tooth movement typically spans 2 to 3 weeks in animal OTM models. Considering the relatively short distance between the tooth and the anchorage point, this study opted to employ elastic bands for OTM. Clinically, patients often find Ni-Ti coil springs less comfortable and may experience more difficulties with oral hygiene during orthodontic treatment.[77]



<**Previous** research OTM model : coil spring>

<**This** research OTM model : elastic band>

Figure 16. OTM model using coil spring and elastic band

4.7.2.2. Orthodontic tooth movement

The appropriate transmission of forces to teeth, and then, alveolar bone underpins OTM. Researcher investigating OTM often choose to work with rat molars, primarily due to the similarity in tooth formation and morphology between rats and humans. In this study, the distance of OTM was measured on days 5, 7, 10, and 14. The OVX group exhibited a continuous increase in tooth movement throughout the experimental period. In contrast, the ROMO group did not show significant differences initially compared to the OVX group but exhibited greater tooth movement early on. However, from day 10 onwards, the ROMO group showed a rapid decrease in tooth movement, ultimately leading to a statistically significant difference on day 14. When ovariectomy is performed on rats, as in the OVX group, it results in hormonal imbalances that induce osteoporosis in the alveolar bone. Estrogen deficiency due to ovariectomy shortens the lifespan and impairs the function of osteoblasts and osteocytes while extending the lifespan of osteoclasts, thus, promoting their differentiation and resorption activity, and ultimately increasing bone turnover.[81,82] This phenomenon likely accelerated tooth movement in the OVX group. In contrast, in the ROMO group, the initial administration of anti-sclerostin accelerated OTM, but this effect was not sustained, and from day 10 onwards, tooth movement decreased. By day 14, the ROMO group exhibited less tooth movement than the OVX group. The hypothesis that romosozumab

administration would initially accelerate OTM, but then decrease it later was partially confirmed, as the differences in OTM were only observed during the later stages of the experiment. When comparing the groups with and without drug administration, no difference was observed in the first week, but from the second week onward, OTM was reduced in the group with drug administration, This finding is consistent with our results. Furthermore, over the years, bisphosphonates, a representative ARD, have been used as inhibitors of OTM in ovariectomized rats. Administering bisphosphonates from the beginning has consistently proven to reduce OTM, which is different from our findings.[21,22,45]. In conclusion, our results demonstrate that romosozumab led to changes in OTM, which may be of clinical significance in orthodontic treatment.

4.7.3. Micro-CT

Micro-CT has been used to assess the three-dimensional microstructure of alveolar bone, allowing for the evaluation of trabecular and cortical bone morphology in animal specimens during the OTM process. In this study, the apical region of the maxillary first molar was observed for biological changes during OTM.[6,12,13,46] High-resolution micro-CT enables direct measurements of specimens three-dimensionally, thereby providing accurate, reproducible, and measurable bone parameters that offer high-quality information about bone mass and structure.[83]

In this study, romosozumab was administered to the ROMO group 1 week after ovariectomy. This time point was selected because it is known to coincide with the degenerative changes in bone.[71-73] Micro-CT analysis revealed significant differences between the group receiving romosozumab and the OVX group in BV/TV, Tb.Th, Tb.N, and Tb.Sp. Specifically, BV/TV and Tb.Th increased, while Tb.N increased and Tb.Sp decreased in the group that received romosozumab during OTM compared to the group that did not. Scl-Ab exhibits a dual effect, initially increasing bone formation, and later, reducing bone resorption, thereby improving bone architecture and mass.[8] This results in a positive bone balance in

remodeling areas that leads to bone formation. Most parameters did not show significant differences on day 5 but differences between groups were noted from day 7. On day 14, the OVX group, which had only undergone ovariectomy, showed the highest bone loss rate (43% lower than those of the ROMO group). The modulation of bone parameters led to improved bone strength. Thus, our results support that Scl-Ab stimulates bone turnover, normalizes bone mass, and maintains or enhances bone quality. The administration of Scl-Ab not only reduced BV/TV in the furcation,[12] but also improved bone strength and mass. These findings are consistent with results from previous animal studies.[66,84]

4.7.4. TRAP

TRAP-positive cells originate from the hematopoietic stem cell lineage and are commonly found in osteoclasts. They play a role in intracellular collagen degradation, leading to the secretion of extracellular matrix, which promotes osteoclast adhesion and migration. Therefore, TRAP-positive cells are considered a useful marker in bone remodeling.[85,86] In this study, TRAP-positive cells decreased in the group treated with romosozumab. This indicates that the inhibition of sclerostin in animals with induced osteoporosis affects osteoclasts and bone resorption.[7,8] Previous research has shown that ARDs, such as alendronate, reduce the proportion of osteoclasts and indirectly inhibit bone formation.[45,87] Similarly, in this study, romosozumab, an anabolic agent, also led to differences between the groups. Our findings were consistent with the similar results of previous studies. In ovariectomized rats, romosozumab is thought to inhibit osteoclastic apoptosis and reduce bone resorption.

4.7.5. IHC

Tumor necrosis factor (TNF) and its related ligand, RANKL, are involved in osteoclast precursor cell surface expression. They bind to its receptor, RANK, on hematopoietic osteoclast precursors, promoting their rapid differentiation into mature osteoclasts. OPG, another member of the TNF family, acts as a decoy receptor produced by osteoblasts. It interferes with the binding of RANK with RANKL.[88] In this study, it was observed that bone remodeling and bone resorption markers in the ROMO group decreased from the 5th day of OTM. Scl-Ab regulates negative regulators of the Wnt signaling pathway, such as sclerostin and DKK1, and activates Wnt signaling to increase OPG, thus inhibiting osteoclast activity and promoting bone formation.[89] Additionally, it affects osteocytic expression related to osteoclastogenesis regulation, ultimately reducing bone resorption.[8,90] Osteoclasts are stimulated and differentiated through the RANKL, RANK, and OPG pathways. Therefore, the ratio of OPG expression to RANKL expression is related to osteoclastogenesis.[91] An increase in RANKL expression and a decrease in OPG expression favor rapid bone resorption, while a decrease in RANKL expression and a higher ratio of OPG expression promote bone formation.[91] In this study, it was observed that not only TRAP-positive cells but also the level of RANKL expression decreased in a similar pattern. This change is consistent with

previous research indicating that the administration of anti-sclerostin agents regulates the ratio of RANKL and OPG expression in osteocytes.[12]

Wnt proteins activate the Wnt/β-catenin signaling pathway by binding to a complex of low-density lipoprotein receptor-related proteins (LRP) 5 and 6 and Wnt-Frizzled (FZD). This activation promotes osteogenesis and reduces bone resorption. The activation of the Wnt signaling pathway is essential for osteoblast maturation and the survival of osteoblasts and osteocytes. The increased survival of osteoblasts and osteocytes leads to increased RANKL and OPG expressions, thus inhibiting osteoclast formation. However, sclerostin, which is targeted in osteoporosis treatment, can bind to LRP 5 and/or 6, thus acting as an extracellular inhibitor of Wnt signaling. Sclerostin inhibits Wnt/β-catenin signaling by interfering with the binding to Wnt-FZD, thereby suppressing osteogenesis.[10] Sclerostin is primarily secreted by osteocytes and is closely related to bone formation and bone mass. It acts as an indirect stimulant for bone resorption by inducing RANKL expression in pre-osteoblasts and osteoblasts while reducing OPG, thereby activating osteoclasts. Additionally, it inhibits Wnt/β-catenin signaling, suppressing osteoblast function and promoting osteoclastogenesis. Therefore, overexpression of SOST/sclerostin leads to a decrease in bone mass.[92] Sclerostin is observable in osteocytes immunohistochemically.[93] When the number

of sclerostin-stained osteocytes was measured, particularly on the compression side, it was confirmed that sclerostin was not expressed in osteoblasts or lining cells. In this study, the number of sclerostin in the ROMO group was significantly lower than in the OVX group. Our finding was consistent with previous research where injecting sclerostin protein accelerated OTM while upregulating RANKL and downregulating OPG.[12] Scl-Ab increases bone mass, improves bone structure, and decreases sclerostin.[8,94] This suggests a negative correlation between increased bone mass and bone strength, and decreased sclerostin, which was also observed in this current study.

Romosozumab, which inhibits sclerostin, promotes bone formation through a modeling-based mechanism and increases bone mass, and trabecular and cortical bone volumes. The inhibition of sclerostin activates the Wnt signaling pathway, thereby increasing the expression of OPG. Since OPG blocks the functional binding of RANKL and RANK, theoretically, an increase in the OPG/RANKL ratio signifies a reduction in osteoclasts. In our ROMO group, a decrease in sclerostin suggests bone formation activation. Moreover, a high OPG/RANKL ratio indicates that bone formation is promoted, while bone resorption is inhibited with romosozumab. However, since the OPG/RANKL ratio influences both bone remodeling and bone modeling, it is difficult to attribute the changes solely to

remodeling, especially as romosozumab primarily acts on modeling-based bone formation. Both bone modeling and remodeling are influenced by osteocytes, osteoblasts, and osteoclasts. The difference is that bone modeling does not involve simultaneous resorption and formation activities, whereas bone remodeling is a local process where new bone formation replaces old bone following resorption.[95] Histological analysis revealed a higher OPG/RANKL ratio in the ROMO group, and radiographic analysis showed positive results for various parameters, although OTM was inhibited after a certain point. Previous studies indicated that Scl-Ab increased the proportion of surfaces with fluorochrome labels corresponding to both remodeling-based and modeling-based bone formation, contributing to an increased bone volume.[96] However, after a certain period, bone formation at the remodeling sites did not increase, implying that Scl-Ab does not promote remodeling beyond a certain point. It seems that in the initial stages of OTM in the presence of romosozumab, both modeling and remodeling processes might occur, potentially affecting bone volume and mass positively. However, after a certain point, it appears that the action is primarily based on modeling, possibly leading to bone formation.

On the compression side, bone remodeling occurs due to mechanical forces, leading to bone resorption, which requires the activation of osteoclasts and is

influenced by a relative increase in RANKL. This affects the rate of OTM. On the tension side, bone formation is stimulated, necessitating the activity of osteoblasts. Consequently, the expression of OPG is promoted. In the ROMO group, due to the indirect effects of romosozumab, the OPG/RANKL ratio was relatively higher. This finding reflects the distinct mechanisms of action on different sides of the tooth, illustrating the complex interplay of bone cells and signaling molecules in response to orthodontic forces and pharmaceutical intervention.

4.8. Limitations

This study examined the relationship between romosozumab and OTM. However, the dosage, administration method, and orthodontic treatment are limited to ensure ethical animal experimentation. Hence, the most effective timing, method, and dosage for optimizing orthodontic treatment remain unclear. As adults of various age groups may be taking different drugs at varying dosages, it is important to consider the effects of each drug on tooth movement. Osteoporosis medications exhibit diverse mechanisms and effects. Understanding these mechanisms may improve orthodontic treatment success.

This study aimed to investigate the effects of romosozumab, a new class of osteoporosis medication, on tooth movement. We did not include non-ovariectomized rats for comparison. Although we demonstrated the effects of romosozumab in ovariectomized rats, it is unclear whether the values obtained are within the normal ranges compared with those in normal rats. Thus, it seems necessary to conduct future studies that compare ovariectomized and normal rats.

V. CONCLUSION

The administration of romosozumab initially accelerated tooth movement in the early stages but later inhibited tooth movement. As new alveolar bone formed, the micro-CT parameters improved. In the ROMO group, bone remodeling markers and sclerostin decreased, but the OPG/RANKL ratio became higher.

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국문요약

난소절제술을 시행한 골다공증 유도 백서에서 로모소주맙이 교정적 치아 이동에 미치는 영향

연세대학교 대학원 치의학과

안 현 나

지도 교수: **박 원 서**

서론

성인 치아 교정 치료의 수요가 최근 몇 년간 지속적으로 높아지고 있다. 특히 중장년층은 치주에 문제가 있거나 전신질환을 가지고 있을 수 있는데, 그중 골다공증은 골밀도가 감소하면서 뼈의 강도가 약해지는 골격 전신질환으로 무시할 수 없는 질병으로 자리 잡고 있어 교정치료 시 주의해야 한다. 골다공증 치료 약물 중 로모소주맙 (romosozumab)은 sclerostin에 결합하여 그 작용을 억제함으로써 초기에는 골형성을 촉진시키고 후기에는 일시적으로 골흡수를 억제시키는 이중 효과를 보이는

최초의 anabolic medication이다. 그러나 새로운 뼈의 형성을 필요로 하는 치아 교정 치료에 대한 약물의 안정성과 부작용 등에 대한 결과를 찾아보기 어려워 이에 대한 연구가 필요해 보인다.

본 연구의 목적은 난소절제술로 유도된 골다공증 동물 모델에 sclerostin antibodyies (Scl-Ab)를 전신 투여가 교정적 치아 이동에 미치는 영향을 평가하는 것이다.

방법

24 마리의 12 주령 암컷 Sprague-Dawley 백서를 무작위로 두 그룹으로 나누어졌다: 1) OVX (난소절제술) 군, ROMO (난소절제술 + 로모소주맙) 군. 모든 그룹에 난소절제술을 한 뒤 8 주의 기간을 주어 골다공증을 유발하였고, ROMO 군에는 난소절제술 2 주 후부터 romosozumab 을 주 2 회 피하주사하고, OVX 군에는 동일한 양의 식염수를 투여하였다. 난소절제술 8 주 후 교정적 치아 이동을 위해 50g 의 힘을 측정하여 제 1 대구치와 미니스크류 사이에 교정용 고무밴드를 연결하였다. 이후 백서를 5, 7, 10 그리고 14 일 차에 희생하였다. 실험 전과 희생 전에 인상채득을 하여 석고 모델을 제작한 뒤 모델 스캔하여 치아 이동량을 계측하였고, micro-computed tomography (micro-CT) 분석, tartrate-resistant acid

phosphatase (TRAP) 염색과 immunohistochemistry (IHC) 분석을 통해 치주조직에 미치는 영향을 평가하였다.

결과

7 일차에 ROMO 군에서 더 많은 치아 이동을 관찰하였으나, 10 과 14 일에는 상대적으로 적은 이동이 있었다. 상악 제 1 대구치의 치근분지부를 분석한 결과, ROMO 군에서 7 일차부터 BV/TV, BV/Th 그리고 Tb.N 이 유의하게 증가하였고, Tb.Sp 가 유의하게 감소하였다. TRAP-positive cells 은 OVX 군에서 더 많이 관찰되었으며, ROMO 군에서 receptor activator of nuclear factor-kappa β ligand (RANKL), osteoprotegerin (OPG) 그리고 sclerostin 의 양성 발현 정도가 확연하게 감소하였다. OPG/RANKL ratio 는 ROMO 군이 OVX 군보다 더 높은 것으로 나타났고, 두 그룹 모두 긴장측이 압박측보다 더 높았다.

결론

Romosozumab의 투여가 실험 초기에 일시적으로 치아 이동을 가속화시키다가 실험 후기에 상대적으로 억제되는 양상을 보였다. ROMO 군에서 새로운 치조골이 형성되면서 micro-CT parameter들이 개선되었고,

bone remodeling markers와 sclerostin은 감소하였으며, OPG/RANKL ratio은 더 높게 측정되었다.

핵심되는 말: 골다공증; 골다공증 약물; 스클레로스틴 항체; Wnt 신호전달 경로; 교정 치료