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Inhibitory effects of 4-hexylresorcinol on root resorption induced by excessive orthodontic force in rat

쥐에서 과도한 교정력 부여 시 4-hexylresorcinol의 치근흡수 억제효과에 관한 연구

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장 준 규

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-ABSTRACT-

Inhibitory effects of 4-hexylresorcinol on root resorption induced by excessive orthodontic force in rat

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Introduction: Root resorption during orthodontic tooth movement (OTM) is caused by imbalance between bone turnover rate and applied mechanical stress. The administration of 4-hexylresorcinol (4HR) increases bone turnover rate and factors associated with bone formation. Thus, 4HR may show protective activity against root resorption by excessive orthodontic force. The objective of this study is to demonstrate effects of 4HR administration on root resorption by excessive orthodontic force through imaging and histological examination.

Material and Methods: A total of 40 rats (male: 20; female: 20) were included in this study and the mandibular first molar was subjected to

excessive orthodontic force. The experimental group (n = 20) received 12.8 mg/kg of 4HR every 2 weeks. The controls (n = 20) received a solvent without 4HR. Both groups had the same sex distribution. On Day 28 after the initiation of OTM, all the animals were sacrificed for micro-computed tomography analysis, western blot analysis and immunohistochemistry.

Results: The ratios of the root length and root volume to the total volume were significantly higher in the experimental group compared to those in the control group ($p < 0.05$). The expression levels of OPG, RANKL, alkaline phosphatase and Runx2 in the experimental group according to western blotting were significantly higher compared to those in the control group ($p < 0.05$). Their expression was mainly found in the periodontal ligament area.

Conclusion: The administration of 4HR decreased root resorption caused by excessive orthodontic force and increased the expression levels of OPG, RANKL, alkaline phosphatase, and Runx2.

Keywords: animal model; 4-hexylresorcinol; root resorption; receptor activator nuclear factor kappa-B ligand(RANKL); Osteoprotegerin(OPG); bone remodeling; bone turnover

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I. INTRODUCTION

Orthodontic tooth movement (OTM) can be achieved by remodeling process of periodontal tissue including cementum and alveolar bone. When orthodontic force is applied, the pressure induces osteoclastic mineralized tissue degradation on the compression side and mineralization by osteoblast on the tension side.[1] This mineralized tissue turnover is the coupling mechanism of bone resorption followed by bone formation and is known as a marker that influences OTM.[2]

Alveolar bone remodeling is influenced by localized tooth-related factors.[3] Systemic factors such as menopause,[4] aging,[5] osteoporosis,[6] changes in parathyroid hormone levels,[7] as well as high levels of steroid[8] or bisphosphonate intake can also influence the overall alveolar bone turnover rate.[9] It is well known that hindered alveolar bone turnover rates can impede the overall velocity of tooth movement during orthodontic treatment.[10]

Certain levels of tooth root resorption are an unavoidable consequence caused by orthodontic forces and there have been many

efforts to minimize it.[11-14] Unfortunately, severe resorption, defined as a reduction that is more than 4 mm or a third of the original length of the root, occurs in 1% to 5% of treated teeth.[11] The etiology of root resorption is multifactorial; there are patient-related factors such as genetic predisposition,[12] treatment-related factors including extensive treatment duration or tooth movement,[13] and local factors of the tooth itself.[14] However, none of these factors have been clearly proven as the principal cause of root resorption.[11] Bone metabolism not only affects the acceleration of tooth movement but also modifies the periodontal tissue and therefore the extent of root resorption.[15] A low bone turnover rate is considered to be a predisposing factor for root resorption and high bone turnover is associated with less root resorption.[16] Moreover, studies have shown that periodic vibration[17] and low level laser therapy (LLLT)[18] stimulation during orthodontic treatment can increase the rate of bone turnover and thus reduce the extent of root resorption. In the case of an ovariectomized mouse model, a lack of estrogen induces the imbalance of the receptor activator of the NF-κB ligand (RANKL)/osteoprotegerin (OPG) system and increases root resorption during OTM.[19] The application of resveratrol in the rat model decreases root resorption during OTM but reduces the amount of OTM.[20] The agent for reducing root resorption during OTM should not inhibit OTM. However, this type of agent is rare.

4-hexylresorcinol (4HR), a phenol derivative with anti-septic, anti-parasitic properties, has been used as a food preservative and as a means to prevent melanosis.[21] It is known to be safe and effective in topical applications for infected skin or mucosa.[21] 4HR suppresses tumor necrosis factor-α (TNF-α)[22] and nuclear factor-κB (NF-κB) signaling pathway[23] related to osteoclast differentiation which increases bone formation by suppressing osteoclast activity and promoting angiogenesis.[24] 4HR has been incorporated into bone-graft material and has been proven to prevent the formation of foreign-body giant cells[25] and suppress NF-κB signaling pathway in osteoblast.[26] Furthermore, it is known to activate transforming growth factor-β1 (TGF-β1) signaling which induces bone regeneration and remodeling.[27] TGF-β1 activated by 4HR induces angiogenesis independently of hypoxia inducible factor (HIF).[24, 28]

In the tooth-related research, 4HR increases bone turnover markers in the blood samples and accelerates OTM.[29] The administration of 4HR increases mineral deposition during tooth formation and mandibular incisor eruption in rat models.[30] Both bone turnover and mineral deposition during tooth formation are important for preventing root resorption during OTM. However, not much is known about its effects on root resorption during OTM. The objective of this study is to evaluate

the effects of 4HR on root resorption by excessive orthodontic force through imaging and histological examination.

II. REVIEW OF LITERATURE

1. 4HR

4HR has been used to treat sore throats due to its anti-parasitic and anti-septic effects. It has also been used as food additives and cosmetic ingredients for its anti-tyrosinase and anti-oxidant properties. Recently 4HR has exhibited various beneficial properties as an ingredient in biomaterials. It is a useful and effective pharmacological chemical that can be used together with other biomaterials in tissue engineering for bone, vessel, and epithelial tissue regeneration[21]. In orthodontic field several studies have shown that 4HR accelerate OTM[29] and eruption rate.[30]

1.1. Characteristics of 4HR

4HR is a substituted phenol, also known as hexylresorcinol, 4-hexyl-1,3-dihydroxybenzene, and 4-hexyl-1,3-benzenediol. 4HR belongs to the class of resorcinols (1,3-Benzenediol) and its linear formula is CH3(CH2)5C6H3-1,3-(OH)2. The molecular weight of 4HR is 194.27012 g/mol. The molecular structure of 4HR is given in Figure 1.

1.2. Use of 4HR

4HR has been widely used in lotions, sprays, and lozenges. It has been used as a topical antiseptic to help prevent skin infection or as a local anesthetic for the relief of a sore throat and its associated pain for decades. 4HR, as an inhibitor of tyrosinase, has been used as a food additive to prevent melanosis in shrimp.[31, 32] 4HR also prevent food from browning during storage of apples by high affinity for polyphenol oxidase.[33] 4HR has also been used as an additive in anti-aging creams[34] for its antioxidant property.

Recently, 4HR has been used with various biomaterials, including bone substitutes, silk, dental implants, and polymers.[21] 4HR incorporated into GBR membrane shows more bone formation in the rabbit calvarial defect than commercial membrane.[35] 4HR also showed anticancer effect when used with cisplatin.[36]

1.3. Relation between 4HR and bone metabolism

4HR inhibits activation of the TNF-α-induced NF-κB signaling pathway through NF-κB phospholuation.[23] NF-κB pathway are important for the development, and differentiation of osteoclast. Inhibition of NF-κB by 4HR is an effective approach to inhibit osteoclast formation and bone resorptive activity.[37] The inhibition of the NF-κB signaling pathway induced by 4HR increases the level of bone formation

marker.[26] 4HR activate TGF-β1 signaling,[28] which induces bone regeneration and remodeling. The osteogenesis-related proteins were expressed in a stage-specific manner in 4HR-treated Saos-2 cells in accordance with the sequence of bone formation induction, osteoblast differentiation, osteoid matrix deposition, and bone maturation.[29]

1.4 Relation between 4HR and tooth movement

Bone turover is an important factor for the rate of eruption and OTM. Since 4HR administration affects bone turover markers,[29] 4HR administration can be expected to affect OTM and tooth eruption. According to recent study, the administration of 4HR in ovariectomized rats increased the rate of OTM and the levels of both bone formation and bone resorption markers.[29] 4HR administration also increased in eruption rate of incisors and expression of markers related to dental hard tissue formation such as TGF-β1, osterix, dentin sialophosphoprotein, parathyroid hormone-related protein receptor, BMP-2/4, and Runx2in rats.[30]

2. RANKL/RANK/OPG system and root resorption

The RANKL/RANK/OPG axis affect not only bone remodeling but also

orthodontically induced apical root resorption.[15] The binding of RANKL to its receptor RANK triggers osteoclast precursors to differentiate into osteoclasts.[38] OPG plays an important role in bone metabolism as a decoy receptor for RANKL in the RANK/RANKL/OPG axis, inhibiting osteoclastogenesis and bone resorption.[39] RANKL and OPG in periodontal ligament are important for bone remodeling and root resorption during OTM.[40]

Odontoclast is a multinucleated cell similar to osteoclast and is present in the resorption lacunae on the root surface. The cellular mechanisms of root resoption appear to be quite sililar to those of osteoclastic bone resorption.[41, 42] During OTM, RANKL expression increases on the compression side, and RANKL activates osteoclastogenesis.[43, 44] On the tensile side, the production of OPG is increased and the release of RANKL from osteoblast is decreased.[45] RANKL-to-OPG ratio in the periodontal ligament seems to be an important for root resorption during OTM.[40] Low level of OPG led to root resorption via increased activation of osteoclasts and reduced mineralization of cementum.[46]

3. Chemicals or medicines that reduce root resorption

3.1 Bisphosphonate

Bisphosphonate binds to hydroxyapatite on the resorptive surface, interferes with the inhibition of osteoclast recruitment and activity, and inhibits bone resorption by a mechanism that leads to osteoclast death by apoptosis.[47] It has been reported that when non-N containing bisphosphonate is administered, the production and release of pro-inflammatory molecules and prostaglandin E2 (PGE2) are inhibited[48] and the root resorption and tooth migration are reduced by suppressing the osteoclast action.[49, 50] Ovariectomized rats have been used as a good osteoporotic model,[51] and estrogen reduction due to ovariectomy increases osteoclastogenesis.[52] Systemic administration of zoledronic acid (N-containing third generation bisphosphonate) significantly reduced root resorption in ovariectomized rats.[53] Bisphosphonate administration has the advantage of reducing root resorption during orthodontic treatment, but it greatly hinders tooth movement.[54]

3.2 PGE 2, NSAID and Steroid

Nonsteroidal anti-inflammatory drugs (NSAID) exhibit antipyretic, analgesic, and anti-inflammatory effects by inhibiting the synthesis of PGE2. PGE2 is strongly related to bone resorption, inhibits production of osteoprotegerin and stimulates production of RANKL.[55] Administration of PGE2 stimulate bone resorption and increase rate of OTM.[56, 57] However,

various studies show conflicting results on the effect of PGE2 administration on root resorption. There are studies proving that the administration of PGE2 does not worsen root resorption,[58] while other studies confirm that it does in fact cause root resorption.[59]

Various research results exist on the effect of NSAID on root resorption. However the effect of NSAID on root resorption also has yet to be clarified. It is believed that some types of NSAIDs and steroid reduce root resorption, while some types have little effect.[60, 61] Results from existing studies have shown that steroids and NSAIDs can impair OTM.[54, 62] Steroid and NSAID can reduce root resorption, but may hinder OTM.

3.3 Lithium

Lithium is one of most widely used medication for mood stabilizer and is highly effective for treating manic disorder such as bipolar depression.[63] Lithium may accelerate bone regeneration and inhibit ostoeclastogenesis by promoting the Wnt/β-catenin pathway.[64, 65] Downregulation of Wnt causes root resorption.[66] In animal experiments with rats, lithium decreased root resorption.[67] However, as the concentration of lithium increased, the rate of tooth movement decreased.

3.4 PTH

Parathyroid hormone (PTH), also called parathormone or parathyrin, is a hormone secreted by the parathyroid glands that modulate calcium and phosphate homeostasis through its effects on bone, kidney, and intestine. PTH has paradoxical effects on bone metabolism. Continuous administration of PTH mainly produces a catabolic effect, whereas the intermittent administration has mostly an anabolic effect.[68-70] Continuous elevation of PTH leads to bone loss, while intermittent short elevations of the hormone level can be anabolic for bone and periodontal tissue.[71] Continuous administration of exogenous PTH induced a significant stimulation of the rate of OTM, but intermittent injections of PTH(1-34) did not accelerate tooth movement.[70]

Long-term intermittent injection of PTH (1-34) facilitated repair of periodontal tissue and root resorption after orthodontic tooth.[72-74] Rat models show that intermittent PTH stimulates both osteoclastogenesis and osteoblastogenesis.[7] This elevating bone turnover rate can accelerate tooth movement without adverse effect on root resorption.

III. MATERIAL AND METHODS

1. Preparation of rats and orthodontic appliance installation

A total of 40 rats (Sprague Dawley) were provided by an animal supplier (Orientbio Inc., Seongnam, Korea). Half were male, and the others were female. All the rats were specifically infection free and 8-weeks old. The subsequent experimental procedure was approved by the Institutional Animal Care and Use Committee of Gangneung-Wonju National University (GWNU-2020-16). Forty rats (2-3 rats per cage) were housed under a 12-h light/12-h dark cycle in a controlled environment at 20-22 ℃ and 40% humidity for one week for acclimation prior to experimentation. The rats had free access to food and water and were all fed a control semisynthetic diet according to a classical recommendation (74% carbohydrates from soybean vegetable oil, 14% proteins from casein, supplemented with a standard vitamin and mineral mix).

All rats received an orthodontic appliance consisting of an 8-mm nickel-titanium closed coil spring (Jinsung, Seoul, Korea) stretched between the right mandibular first molars and right mandibular incisors

(Figure 2). The ring connected to the spring was inserted onto the incisor and fixed with light-cured resin. The other side of the spring was ligated to the first molar. Indentation was prepared with a sharp diamond bur on the distal surface of the right mandibular first molar for preventing wire loss. A ligature wire was passed through this indentation. The force initially generated by the coiled spring was 120 g, as measured with the use of force gauge. The appliance was left in place without reactivation for 4 weeks to induce OTM of the mandibular first molar.

2. Experimental design

There were two groups, and each group had 20 rats (10 males and 10 females); as a control, solvent without 4HR was used. The rats in the experimental group received 12.8 mg/kg of 4HR. The injection of 4HR was performed every 2 weeks. An injection agent was prepared just before injection, and the agent was injected into the back skin subcutaneously. The deactivation of the coiled spring was checked with regard to a predetermined schedule. At 4 weeks after appliance application, blood was sampled from the heart under general anesthesia. Then, all the animals were sacrificed humanely.

The following animals were excluded for further analysis: (1) animals showing a loss of the orthodontic appliance during follow-up, and (2) those in which the gap formed by OTM was less than 0.5 mm. A summary of the exclusion process is shown in the flowchart (Figure 3). A biopsy of the mandible was also performed. Histological and molecular biology analyses were conducted.

3. Detection of root resorption using plain X-ray and micro-computed tomography (micro-CT)

Both mandibles were collected, and plain X-rays were taken using an intra-oral radiogram sensor. The lengths of four roots in the first mandibular molar were measured using SigmaScan Pro (SPSS Inc., Chicago, IL, USA). The central two roots were interposed in a plain X-ray, and the outermost border was used for comparison. The ratio for each root length between the affected side and the non-affected side was calculated.

The hemi-mandibles were sent to Genoss (Suwon, Korea) and the Korea National University of Transportation (Chungju, Korea) for analysis with micro-CT. Hemi-mandibles were loaded on the micro-CT scanner (SkyScan1173, Bruker, Kontich, Belgium). The source voltage was 130 kV, and the image pixel size was 13.85 μm. Three-

dimensional reconstruction was performed with the subtraction of the surrounding bony structure. The total volume (TV) and root volume (RV) of the mandibular first molar were measured. The ratio of RV to TV was calculated and compared between groups.

4. Immunohistochemical determination

To assess the expression of OPG, RANKL, alkaline phosphatase (AP), and runt-related transcription factor 2 (Runx2) in the mandible samples, we performed immunohistochemical staining using anti-OPG, anti-RANKL, anti-alkaline phosphatase, and anti-Runx2 antibodies (Santa Cruz Biotech, Santa Cruz, CA, USA). A silane-coated glass slide was used for preventing tissue detachment during the staining procedure. After hydration, sections were treated with trypsin for 5 min. Then, 30% H_2O_2 was applied on the sections for 7 min. After washing with phosphate buffered saline (PBS) twice, protein blocking was performed for 1 h with a ready-to-use solution (Serum-Free Protein Block, Dako North America Inc., Carpinteria, CA, USA). Then, primary antibodies (dilution ratio 1:100) were applied on the tissue section. Incubation with a primary antibody was performed at 4 ℃ overnight. Three washes were performed with PBS. A universal secondary antibody (Dako REAL™ EnVision™/HRP, Rabbit/Mouse; Dako North America Inc.) was conjugated under a humid chamber. Three washes were performed with PBS again. After removing the unreacted secondary antibody, the slides

were stained with a chromogen (Dako REAL™ DAB+ Chromogen and Dako REAL™ Substrate Buffer; Dako North America Inc.).

5. Western blot analysis

The other mandible samples (n = 5 for each group) were collected in a cryovial and stored in a deep freezer (−70 ℃). For the extraction of tissue protein, frozen tissue was crushed and mixed with a tissue-protein-extraction reagent buffer with a protease inhibitor cocktail. Subsequently, western blot analysis was performed.

Proteins were collected and mixed with a sodium dodecyl sulfate buffer. After heat denaturation, they were electrophoresed on 10% polyacrylamide gels. The gels were transferred to polyvinylidene difluoride membranes. After blocking, the membranes were probed with primary antibodies (dilution ratio = 1:500). Blots were imaged and quantified using a ChemiDoc XRS system (Bio-Rad Laboratories Hercules, CA, USA).

6. Statistical analysis

Shapiro-Wilk' s test and Levene' s test were used to assess for normality and homogeneity, and subsequently the comparison between groups was performed with the independent-samples t-test. The level

of significance was set at <0.05. The data were processed with SPSS software (SPSS Inc., Chicago, Il, USA).

IV. RESULTS

1. Application of 4HR and root resorption

1.1 The ratio of root length in periapical radiograph

The ratios of the root length at 4 weeks after OTM in the male control group were 0.94 ± 0.08, 0.81 ± 0.10, and 0.81 ± 0.10 in the distal, central, and mesial roots, respectively (Figure 4). Those in the male experimental group were 1.00 ± 0.09, 0.95 ± 0.11, and 0.99 ± 0.06 in the distal, central, and mesial roots, respectively. The difference between groups was statistically significant in the central and mesial roots (p = 0.017 and 0.001, respectively).

The ratios of the root length at 4 weeks after OTM in the female control group were 0.94 ± 0.06, 0.82 ± 0.03, and 0.76 ± 0.06 in the distal, central, and mesial roots, respectively. Those in the female experimental group were 1.00 ± 0.07, 0.95 ± 0.08, and 0.98 ± 0.09 in the distal, central, and mesial roots, respectively. The difference between groups was statistically significant in the central and mesial roots (p = 0.005 and 0.001, respectively).

1.2 The root morphology and RV/TV in micro-CT

The results of the micro-CT analysis were in accordance with the results for plain film (Figure 5). Three-dimensionally reconstructed images demonstrated that root resorption was severe in the central roots. Multiple lacunas were observed on the surface of the mesial root in the high-magnification view. These lacunas were not confined to the surface of the compression side.

The RV and TV were calculated from the micro-CT, and their ratio was compared between groups (Table 1). The comparison of the percentages of RV to TV showed a statistically significant difference between groups (p = 0.002 and 0.006 for male and female, respectively).

2. Application of 4HR and bone turnover marker

In the western blot analysis, the expression level of OPG, RANKL, Runx2, and AP were increased in the experimental group (Figure 6). The results of immunohistochemical staining were in accordance with the results of western blot.

2.1. Histologic analysis

Upon histological analysis, the findings for active root resorption were similar in both groups. However, the area of active root resorption was more frequently observed in the control group (Figure 7a). The thickness of the cementum was thinner in the middle area of the root surface. Discontinuous cementum was frequently found in the control group. The results of immunohistochemical staining were in accordance with the results of western blot (Figure 7b). The positive reaction to AP, OPG, RANKL, and Runx2 was mainly found in the periodontal ligament area.

2.2. Western blot

The administration of 4HR increased the expression of OPG, RANKL, Runx2, and AP in both sexes of the rats (Figure 6). The relative expressions of OPG, RANKL, Runx2, and AP in the male control group were 1.00 ± 0.15, 1.11 ± 0.10, 1.12 ± 0.20, and 1.32 ± 0.42, respectively. Those in the male experimental group were 1.90 ± 0.13, 1.78 ± 0.06, 1.83 ± 0.23, and 2.34 ± 0.13, respectively. The difference between groups was significantly different for OPG, RANKL, Runx2, and AP (p = 0.014, 0.009, 0.015, and 0.016, respectively). The RANKL-to-OPG ratio for males was 1.12 ± 0.23 and 0.97 ± 0.04 for the control and experimental groups, respectively ($p > 0.05$).

The relative expressions of OPG, RANKL, Runx2, and AP in the female control group were 0.81 ± 0.04, 0.94 ± 0.13, 1.16 ± 0.75, and 1.52 ± 0.16, respectively. That in the male experimental group was 2.29 ± 0.38, 1.56 ± 0.13, 2.32 ± 0.60, and 2.01 ± 0.84, respectively. The difference between groups was significantly different for OPG and RANKL (p = 0.040 and 0.007, respectively). The RANKL-to-OPG ratio for female was 1.16 ± 0.16 and 0.70 ± 0.15 for the control and experimental group, respectively ($p < 0.05$).

V. DISCUSSION

Root resorption during OTM has been considered an unavoidable phenomenon.[14] However, severe resorption is considered to be a complication and has been reported in 1% to 5% of OTM.[14] Accordingly, reducing root resorption during OTM is an important issue in orthodontics. In this study, the administration of 4HR to rat models resulted in reduced root resorption induced by OTM (Figure 4 and 5). The bone turnover markers AP, OPG, RANKL, and Runx2 showed elevated expression levels in the 4HR administered group (Figure 6 and 7). To the best of our knowledge, this is the first report about reducing root resorption during OTM by 4HR administration.

The small size of rats' molars hindered the ability to use orthodontic brackets. Instead, ligature wire was used to directly tie the NiTi coil springs to the mandibular right first molar. Therefore, mesial tipping movement was induced rather than pure translation of the molars. Mesial tipping leads to the intrusion of the mesial root as well as the extrusion of the distal root. This causes more root resorption to occur in the mesial root compared to the distal and central root.

There was significant difference in the amount of mesial root resorption of the treatment group compared to the control group. In the control group, the length of the mesial root reduced to 80% in males and 76% in females. In the treatment group, the mesial root length measured 99% in males and 97% in females. These results are clinically significant and prove that the administration of 4HR effectively lessens root resorption when teeth are subjected to excessive orthodontic force.

Micro-CT was used to assess the RV/TV ratio of the rats' molars. The results showed only a 2-3% percent difference between the treatment group and the control group. This is because the amount of the central root and distal root resorption was slight in the control group as well as in the treatment group. Thus, the difference in total volume of roots was insignificant. Secondly, the anatomy of a root tapers down at the apex. Even though there was a difference in the length of resorption, the difference in volume was minor.

1. The difference between 4HR and other substances in the effect on root resorption

Several studies have been conducted on the effects of various drugs or chemicals on the rate of tooth movement and root resorption.[75]

Bisphosphonate binds to hydroxyapatite on the absorbing surface of bone and inhibits bone resorption through a mechanism that inhibits osteoclast migration and action and induces osteoclast apoptosis.[47] It has been reported that the administration of bisphosphonate reduces root resorption and tooth movement[50] due to its anti-inflammatory action and inhibition of osteoclastic activity.[48] Bisphosphonate administration has the advantage of reducing root resorption during OTM, but it significantly interferes with OTM.[54] Prednisolone administration also decreases root resorption and the rate of tooth movement.[76] Controversial results have been reported for the effect of NSAID administration on root resorption.[76] It is believed that some types of NSAIDs inhibit root resorption[75] and tooth movement.[65] Lithium promotes reduced osteoclast formation by promoting the Wnt/β-catenin signaling system.[65] As the concentration of lithium increases, root resorption and tooth movement decreases in animal experiments.[67]

As described above, most of the substances administered to reduce root resorption through a mechanism that reduces osteoclast activity have tended to decrease tooth movement speed as well as root resorption.[67] The 4HR used in this study resulted in root resorption being reduced compared to that in the control group (Figure 4 and 5). When looking at the numbers of exclusions because of a low rate of tooth

movement, there were four for the experimental group and five for the control group (Figure 3). The administration of 4HR accelerates OTM in ovariectomized rat models.[29]

The bone remodeling process is balanced through the coupling of bone resorption by osteoclasts and bone formation by osteoblasts. Medications or dietary supplements that decrease osteoclastic activity suppress root resorption but also hinder OTM.[75] The substances that increase bone turnover rate and increase both osteoblastic and osteoclastic activity may diminish root resorption and promote tooth movement. The effect of PTH is different according to the treatment method that is either catabolic or anabolic.[77] The long-term intermittent injection of PTH facilitates the repair of root resorption.[78] Intermittent PTH administration increases the expression levels of both RANKL and insulin-like growth factor-1 (IGF-1), indicating that intermittent PTH stimulates both osteoclasts and osteoblasts.[7] LLLT may inhibit orthodontically induced root resorption[18] but accelerates OTM.[79] LLLT accelerates the bone remodeling process by stimulating both osteoblastic and osteoclastic activity during OTM.[80] 4HR facilitates OTM in ovariectomized rat models.[29] In our study, 4HR increased the bone turnover markers, which were RANKL, OPG, Runx2, and AP, in the experimental group more than

in the control group (Figure 6). An increased bone turnover rate might inhibit root resorption (Figure 4).

Most substances that reduce osteoclastic activity hinder OTM and reduce root resorption. However, substances that increase osteoclastogenesis and osteoblastogenesis simultaneously and substances that initially increase osteoclastic activity but later increase osteoblastic activity have been reported to reduce root resorption without interfering OTM. Since extended orthodontic treatment duration is also a risk factor for root resorption, substances that reduce root resorption but increase the treatment period are not suitable for orthodontic treatments. For root resorption during orthodontic treatment, suitable substances should not hinder OTM at least. Previous studies[29] have shown that 4HR accelerates OTM. In This study we show that 4HR decrease the root resorption, and conclude that 4HR is suitable for orthodontic treatments.

2. Root resorption and RANKL/OPG ratio

The main physiologic role of the RANKL/RANK/OPG system is regulating bone remodeling. Functional osteoclast formation and bone resorption are dependent on the ratio of RANKL/OPG expressed by

osteoblastic cells and RANK expression by osteoclast precursor cells.[81] The RANKL/RANK/OPG system may influence root resorption during OTM, as well.[15] Odontoclasts are multinucleated cells locate on root dentin being resorbed. RANKL expression was detected not only in osteoclasts but also in odontoclasts, so there seems to be a common regulatory mechanism between osteoclasts and odontoclasts.[82] If root resorption is more severe, the expression of RANKL increases.[44] The amount of RANKL is an important factor for root resorption, but the low ratio of RANKL/OPG and the high concentration of OPG are also important for hard-tissue repair. In this study, the expression of both RANKL and OPG may have contributed in inhibiting root resorption in the experimental group (Figure 4 and 5).

Root resorption during OTM is closely associated with the RANKL-to-OPG ratio.[83] In this study, both RANKL and OPG were shown be elevated in their expression by 4HR administration. But the elevated expressions of RANKL is lower than those of OPG in experimental group, RANKL/OPG ratio is lower in experimental group than control group (Figure 6).

Considering that 4HR inhibits the NF-κB pathway,[22, 26] the effects on osteoclasts and cementoclasts by the elevated expression of RANKL are restricted by hampering its downstream signaling and by the blocking of 4HR. TGF-β1 inhibits mineralized tissue resorption by elevating the OPG level.[83] Interestingly, 4HR is a strong inducer of TGF-β1.[21, 84] Cementum is a barrier to root resorption and its resorption is dependent on the RANKL-to-OPG ratio.[83] In this study, the experimental group showed a lower RANKL-to-OPG ratio compared to the control group ($p < 0.05$ for the female group, Figure 6b).

3. Root resorption and orthodontic force magnitude in rat molar

Rats have been used frequently as a model to study OTM, despite the morphological and physiological differences in the periodontal ligament and alveolar bone compared to humans.[85] In this study, the experiment teeth included the lower first molar. Although anterior teeth are known to be the most vulnerable teeth for root resorption,[86] since the anterior teeth of the rats continue to grow, they are not suitable as an experimental model for root resorption. As heavier forces were applied to tooth, greater root resorption occurred.[87] A large force of 120g was used to examine the effect on root resorption in this study. Because a human molar is approximately 20 times larger than a rat molar,[88] the effect of a 120g force on a rat molar is comparable with that of a very

heavy force of 2400g on a human molar. This is a force that is large enough to cause root resorption. In this study, it was found that root resorption induced by a very heavy force was alleviated by 4HR (Figure 4 and 5). Further research is needed to investigate the effect of 4HR on root resorption under normal orthodontic force.

4. Limitations in this study

The limitations of this study were as follows: First, rats have a continuous erupting incisor and no premolar. In addition, the root morphology of the mandibular first molar is much different from that in humans. Accordingly, the direct translation of this result to clinical application should be considered as preliminary.

Second, this study focused on bone turnover markers to interpret the mechanism of 4HR's effects. However, these markers might influence the turnover of other calcified tissue such as cementum. Actually, all the surfaces that showed active root resorption had a broken cemental line (Figure 7a). Secondary cementum has bone-like structures with cementoblasts. Without breaking the cemental line, dentin resorption would be impossible.[89] Interestingly, mineralized tissue formation during tooth eruption is enhanced by 4HR administration according to our recent

research.[30] RANKL/OPG is also involved in the turnover of cementum.[83] In this study, the expression levels of RANKL and OPG were increased by 4HR administration (Figure 6). Therefore, 4HR might increase cementum formation during OTM, and this might be one mechanism of reducing root resorption. In addition, increasing M2-type macrophage is associated with reduced root resorption.[89] 4HR is a potent M2 polarizing agent.[24, 90]

Third, heavy force applied on the root surface might induce an inflammatory reaction and subsequent pH drop. An acidic environment can decalcify mineralized tissue without the help of cells. Direct resorption without cells was not examined in this study. Further investigation for the clinical application of 4HR in orthodontic treatment is necessary.

Fourth, despite the elevation of both bone-formation and bone-resorption markers in the alveolus supports in a previous study,[29] the systemic effects of 4HR injection were not evaluated, and the mechanisms of how 4HR affects the signaling pathway of bone metabolism is still unclear. Further study is necessary to elucidate the in vivo mechanism of 4HR's activity. 4HR has been accorded a GRAS

(generally recognized as safe) status as an oral health care agent.[91] There are some issues for the estrogenic activity of 4HR,[92] but a recent study reported that 4HR behaves differently from other xeno-estrogens.[93]

Fifth, there was no statistically significant difference in the amount OTM between the experimental group and the control group (Table 2). NiTi coil springs were deactivated in both groups after 4 weeks of OTM. If the measurements were made every week rather than after 4 weeks, the accelerated OTM could be observed in the experimental group as in the previous study.[29]

Sixth, tartrate-resistant acid phosphatase (TRAP) staining was not performed to compare the number of osteoclast in the treatment group and the control group in this study. Although the expression of bone turnover markers such as RANKL, OPG, RUNX2, AP were examined, this study fails to find evidence of bone turnover on the cellular level. This is the limitation of this study and further research is needed to develop more concrete results.

VI. CONCLUSION

In this study, the administration of 4HR decreased the root resorption of the mandibular first molar caused by excessive orthodontic force and increased the expression levels of OPG, RANKL, alkaline phosphatase, and Runx2 in the mandible.

During orthodontic treatment, orthodontic force may lead to concentrated pressure on a select number of teeth or roots. This study supports that the administration of 4HR can prevent root resorption. Further studies must be conducted before the clinical application of 4HR can begin to prevent the undesirable side effect of root resorption during orthodontic treatment.

When previous finding and our study were combined, 4HR administration may be a helpful method with which to reduce root resorption and shorten orthodontic treatment duration, especially in patient who are vulnerable to root resorption or when a large amount of tooth movement is required.

VII. REFERENCES

1. King G, Keeling S, Wronski T. Histomorphometric study of alveolar bone turnover in orthodontic tooth movement. Bone 1991;12(6):401-09.

2. Roberts WE, Huja S, Roberts JA. Bone modeling: biomechanics, molecular mechanisms, and clinical perspectives. Semin. Orthod. 2004;10(2):123-61.

3. Tyrovola JB, Spyropoulos MN. Effects of drugs and systemic factors on orthodontic treatment. Quintessence Int. 2001;32(5):365-71.

4. Yamashita-Mikami E, Tanaka M, Sakurai N, Arai Y, Matsuo A, Ohshima H, et al. Correlations between alveolar bone microstructure and bone turnover markers in pre-and post-menopausal women. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 2013;115(4):e12-e19.

5. Misawa Y, Kageyama T, Moriyama K, Kurihara S, Yagasaki H, Deguchi T, et al. Effect of age on alveolar bone turnover adjacent to maxillary molar roots in male rats: a histomorphometric study. Arch. Oral Biol. 2007;52(1):44-50.

6. Sidiropoulou-Chatzigiannis S, Kourtidou M, Tsalikis L. The effect of osteoporosis on periodontal status, alveolar bone and orthodontic tooth movement. A literature review. J. Int. Acad. Periodontol. 2007;9(3):77-84.

7. Li F, Li G, Hu H, Liu R, Chen J, Zou S. Effect of parathyroid hormone on experimental tooth movement in rats. Am. J. Orthod. Dentofacial Orthop. 2013;144(4):523-32.

8. Verhoeven A, Boers M, te Koppele J, van der Laan W, Markusse H, Geusens P, et al. Bone turnover, joint damage and bone mineral density in early

rheumatoid arthritis treated with combination therapy including high-dose prednisolone. Rheumatology 2001;40(11):1231-37.

9. Shoji S, Tabuchi M, Miyazawa K, Yabumoto T, Tanaka M, Kadota M, et al. Bisphosphonate inhibits bone turnover in OPG-/- mice via a depressive effect on both osteoclasts and osteoblasts. Calcif. Tissue Int. 2010;87(2):181-92.

10. Midgett RJ, Shaye R, Fruge Jr JF. The effect of altered bone metabolism on orthodontic tooth movement. Am. J. Orthod. 1981;80(3):256-62.

11. Weltman B, Vig KW, Fields HW, Shanker S, Kaizar EE. Root resorption associated with orthodontic tooth movement: a systematic review. Am. J. Orthod. Dentofacial Orthop. 2010;137(4):462-76.

12. Al-Qawasmi RA, Hartsfield Jr JK, Everett ET, Flury L, Liu L, Foroud TM, et al. Genetic predisposition to external apical root resorption. Am. J. Orthod. Dentofacial Orthop. 2003;123(3):242-52.

13. Segal G, Schiffman P, Tuncay O. Meta analysis of the treatment-related factors of external apical root resorption. Orthod. Craniofac. Res. 2004;7(2):71-78.

14. Sehr K, Bock NC, Serbesis C, Hönemann M, Ruf S. Severe external apical root resorption—local cause or genetic predisposition? J. Orofac. Orthop. 2011;72(4):321.

15. Tyrovola JB, Spyropoulos MN, Makou M, Perrea D. Root resorption and the OPG/RANKL/RANK system: a mini review. J. Oral Sci. 2008;50(4):367-76.

16. Deguchi T, Seiryu M, Daimaruya T, Garetto LP, Takano-Yamamoto T, Roberts WE. Decreased alveolar bone turnover is related to the occurrence of root resorption during experimental tooth movement in dogs. Angle Orthod.

2015;85(3):386-93.

17. Nishimura M, Chiba M, Ohashi T, Sato M, Shimizu Y, Igarashi K, et al. Periodontal tissue activation by vibration: intermittent stimulation by resonance vibration accelerates experimental tooth movement in rats. Am. J. Orthod. Dentofacial Orthop. 2008;133(4):572-83.

18. Suzuki SS, Garcez AS, Suzuki H, Ervolino E, Moon W, Ribeiro MS. Low-level laser therapy stimulates bone metabolism and inhibits root resorption during tooth movement in a rodent model. J. of Biophotonics 2016;9(11-12):1222-35.

19. Amaro ER, Ortiz FR, Dorneles LS, de Souza Santos M, Barrioni BR, Miranda RM, et al. Estrogen protects dental roots from orthodontic-induced inflammatory resorption. Arch. Oral Biol. 2020;117:104820.

20. Liu X-c, Wang X-x, Zhang L-n, Yang F, Nie F-j, Zhang J. Inhibitory effects of resveratrol on orthodontic tooth movement and associated root resorption in rats. Arch. Oral Biol. 2020;111:104642.

21. Kim JY, Seok H. Role of 4-Hexylresorcinol in the Field of Tissue Engineering. Appl. Sci. 2020;10(10):3385.

22. Ahn J, Kim SG, Kim MK, Kim DW, Lee JH, Seok H, et al. Topical delivery of 4-hexylresorcinol promotes wound healing via tumor necrosis factor-α suppression. Burns 2016;42(7):1534-41.

23. Kim SG, Lee SW, Park YW, Jeong JH, Choi JY. 4-hexylresorcinol inhibits NF-κB phosphorylation and has a synergistic effect with cisplatin in KB cells. Oncol. Rep. 2011;26(6):1527-32.

24. Jo YY, Kim DW, Choi JY, Kim SG. 4-Hexylresorcinol and silk sericin increase the expression of vascular endothelial growth factor via different

pathways. Sci. Rep. 2019;9(1):1-11.

25. Kweon HY, Kim SG, Choi JY. Inhibition of foreign body giant cell formation by 4-hexylresorcinol through suppression of diacylglycerol kinase delta gene expression. Biomaterials 2014;35(30):8576-84.

26. Song JY, Kim SG, Park Nr, Choi JY. Porcine bone incorporated with 4-hexylresorcinol increases new bone formation by suppression of the nuclear factor kappa B signaling pathway. J. Craniofac. Surg. 2018;29(7):1983-90.

27. Kim MK, Yoon CS, Kim SG, Park YW, Lee SS, Lee SK. Effects of 4-hexylresorcinol on protein expressions in RAW 264.7 cells as determined by immunoprecipitation high performance liquid chromatography. Sci. Rep. 2019;9(1):1-15.

28. Kim MK, Kim SG, Lee SK. 4-Hexylresorcinol induced angiogenesis potential in human endothelial cells. Maxillofac. Plast. Reconstr. Surg. 2020;42(1):1-11.

29. Choi KH, Kim DW, Lee SK, Kim SG, Kim TW. The administration of 4-hexylresorcinol accelerates orthodontic tooth movement and increases the expression level of bone turnover markers in ovariectomized rats. Int. J. Mol. Sci. 2020;21(4):1526.

30. Kim JH, Kim DW, Kim SG, Kim TW. 4-Hexylresorcinol administration increases dental hard tissue formation and incisor eruption rate in rats. Appl. Sci. 2020;10(16):5511.

31. Guandalini E, Ioppolo A, Mantovani A, Stacchini P, Giovannini C. 4-Hexylresorcinol as inhibitor of shrimp melanosis: efficacy and residues studies; evaluation of possible toxic effect in a human intestinal in vitro model (Caco-2); preliminary safety assessment. Food Addit. Contam. 1998;15(2):171-80.

32. Frankos VH, Schmitt DF, Haws LC, McEvily AJ, Iyengar R, Miller SA, et al. Generally recognized as safe (GRAS) evaluation of 4-hexylresorcinol for use as a processing aid for prevention of melanosis in shrimp. Regul. Toxicol. Pharmacol. 1991;14(2):202-12.

33. Arias E, González J, Peiró JM, Oria R, Lopez-Buesa P. Browning prevention by ascorbic acid and 4-hexylresorcinol: different mechanisms of action on polyphenol oxidase in the presence and in the absence of substrates. J. Food Sci. 2007;72(9):C464-70.

34. Farris P, Zeichner J, Berson D. Efficacy and Tolerability of a Skin Brightening/Anti-Aging Cosmeceutical Containing Retinol 0.5%, Niacinamide, Hexylresorcinol, and Resveratrol. J. Drugs Dermatol. 2016;15(7):863-8.

35. Lee SW, Kim SG, Song JY, Kweon H, Jo YY, Lee KG, et al. Silk fibroin and 4-hexylresorcinol incorporation membrane for guided bone regeneration. J. Craniofac. Surg. 2013;24(6):1927-30.

36. Kim SG, Choi JY. 4-hexylresorcinol exerts antitumor effects via suppression of calcium oscillation and its antitumor effects are inhibited by calcium channel blockers. Oncol. Rep. 2013;29(5):1835-40.

37. Abu-Amer Y. NF-kappaB signaling and bone resorption. Osteoporos. Int. 2013;24(9):2377-86.

38. Park JH, Lee NK, Lee SY. Current understanding of RANK signaling in osteoclast differentiation and maturation. Mol. Cells 2017;40(10):706.

39. Simonet W, Lacey D, Dunstan C, Kelley M, Chang M-S, Lüthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89(2):309-19.

40. Low E, Zoellner H, Kharbanda OP, Darendeliler MA. Expression of mRNA for osteoprotegerin and receptor activator of nuclear factor kappa β ligand (RANKL) during root resorption induced by the application of heavy orthodontic forces on rat molars. Am. J. Orthod. Dentofacial Orthop. 2005;128(4):497-503.

41. Brudvik P, Rygh P. Multi-nucleated cells remove the main hyalinized tissue and start resorption of adjacent root surfaces. Eur. J. Orthod. 1994;16(4):265-73.

42. Kanzaki H, Chiba M, Shimizu Y, Mitani H. Periodontal ligament cells under mechanical stress induce osteoclastogenesis by receptor activator of nuclear factor κB ligand up-regulation via prostaglandin E2 synthesis. J. Bone Miner. Res. 2002;17(2):210-20.

43. Oshiro T, Shiotani A, Shibasaki Y, Sasaki T. Osteoclast induction in periodontal tissue during experimental movement of incisors in osteoprotegerin-deficient mice. Anat. Rec. 2002;266(4):218-25.

44. Yamaguchi M, Aihara N, Kojima T, Kasai K. RANKL increase in compressed periodontal ligament cells from root resorption. J. Dent. Res. 2006;85(8):751-56.

45. Tang L, Lin Z, Li Y-m. Effects of different magnitudes of mechanical strain on osteoblasts in vitro. Biochem. Biophys. Res. Commun. 2006;344(1):122-28.

46. Liu Y, Du H, Wang Y, Liu M, Deng S, Fan L, et al. Osteoprotegerin-knockout mice developed early onset root resorption. J. Endod. 2016;42(10):1516-22.

47. Rodan GA, Fleisch HA. Bisphosphonates: mechanisms of action. J. Clin. Invest. 1996;97(12):2692-96.

48. Liu L, Igarashi K, Haruyama N, Saeki S, Shinoda H, Mitani H. Effects of local

administration of clodronate on orthodontic tooth movement and root resorption in rats. Eur. J. Orthod. 2004;26(5):469-73.

49. Igar K, Adachi H, Mitani H, Shinoda H. Inhibitory effect of the topical administration of a bisphosphonate (risedronate) on root resorption incident to orthodontic tooth movement in rats. J. Dent. Res. 1996;75(9):1644-49.

50. Fujimura Y, Kitaura H, Yoshimatsu M, Eguchi T, Kohara H, Morita Y, et al. Influence of bisphosphonates on orthodontic tooth movement in mice. Eur. J. Orthod. 2009;31(6):572-77.

51. Omi N, Ezawa I. The effect of ovariectomy on bone metabolism in rats. Bone 1995;17(4):S163-S68.

52. Horowitz MC. Cytokines and estrogen in bone: anti-osteoporotic effects. Science 1993;260(5108):626-28.

53. Sirisoontorn I, Hotokezaka H, Hashimoto M, Gonzales C, Luppanapornlarp S, Darendeliler MA, et al. Orthodontic tooth movement and root resorption in ovariectomized rats treated by systemic administration of zoledronic acid. Am. J. Orthod. Dentofacial Orthop. 2012;141(5):563-73.

54. Bartzela T, Türp JC, Motschall E, Maltha JC. Medication effects on the rate of orthodontic tooth movement: a systematic literature review. Am. J. Orthod. Dentofacial Orthop. 2009;135(1):16-26.

55. Nefussi JR, Baron R. PGE2 stimulates both resorption and formation of bone in vitro: differential responses of the periosteum and the endosteum in fetal rat long bone cultures. Anat. Rec. 1985;211(1):9-16.

56. Seifi M, Eslami B, Saffar AS. The effect of prostaglandin E2 and calcium gluconate on orthodontic tooth movement and root resorption in rats. Eur. J.

Orthod. 2003;25(2):199-204.

57. García-Cabral EJ, Sanchez-Najera RI, Mass-Enriquez A, Ramirez-Herrera GM, Leal-Camarillo JN, Treviño-Tijerina MC, et al. Prostaglandin E2, an orthodontic point of view. Int. J. Appl. Dent. Sci. 2019;5(3):176-80.

58. Widayati R, Suniarti DF, Poerwaningsih EH, Iskandriati D. Root Resorption and RANKL concentration in orthodontic tooth movement accompanied by topical PGE2 Gel application. J. Int. Dent. Medical Res. 2016;9(3):228.

59. Brudvik P, Rygh P. Root resorption after local injection of prostaglandin E2 during experimental tooth movement. Eur. J. Orthod. 1991;13(4):255-63.

60. Gonzales C, Hotokezaka H, Matsuo K, Shibazaki T, Yozgatian JH, Darendeliler MA, et al. Effects of steroidal and nonsteroidal drugs on tooth movement and root resorption in the rat molar. Angle Orthod. 2009;79(4):715-26.

61. Verna C, Hartig LE, Kalia S, Melsen B. Influence of steroid drugs on orthodontically induced root resorption. Orthod. Craniofac. Res. 2006;9(1):57-62.

62. Walker JB, Buring SM. NSAID impairment of orthodontic tooth movement. Ann. Pharmacother. 2001;35(1):113-5.

63. Machado-Vieira R, Manji HK, Zarate CA, Jr. The role of lithium in the treatment of bipolar disorder: convergent evidence for neurotrophic effects as a unifying hypothesis. Bipolar Disord. 2009;11 Suppl 2(Suppl 2):92-109.

64. Li L, Peng X, Qin Y, Wang R, Tang J, Cui X, et al. Acceleration of bone regeneration by activating Wnt/β-catenin signalling pathway via lithium released from lithium chloride/calcium phosphate cement in osteoporosis. Sci.

Rep. 2017;7:45204.

65. Arioka M, Takahashi-Yanaga F, Sasaki M, Yoshihara T, Morimoto S, Hirata M, et al. Acceleration of bone regeneration by local application of lithium: Wnt signal-mediated osteoblastogenesis and Wnt signal-independent suppression of osteoclastogenesis. Biochem. Pharmacol. 2014;90(4):397-405.

66. Lim WH, Liu B, Hunter DJ, Cheng D, Mah SJ, Helms JA. Downregulation of Wnt causes root resorption. Am. J. Orthod. Dentofacial Orthop. 2014;146(3):337-45.

67. Ino-Kondo A, Hotokezaka H, Kondo T, Arizono K, Hashimoto M, Hotokezaka Y, et al. Lithium chloride reduces orthodontically induced root resorption and affects tooth root movement in rats. Angle Orthod. 2018;88(4):474-82.

68. Poole KE, Reeve J. Parathyroid hormone - a bone anabolic and catabolic agent. Curr. Opin. Pharmacol. 2005;5(6):612-7.

69. Lombardi G, Di Somma C, Rubino M, Faggiano A, Vuolo L, Guerra E, et al. The roles of parathyroid hormone in bone remodeling: prospects for novel therapeutics. J. Endocrinol. Invest. 2011;34(7 Suppl):18-22.

70. Soma S, Iwamoto M, Higuchi Y, Kurisu K. Effects of continuous infusion of PTH on experimental tooth movement in rats. J. Bone Miner. Res. 1999;14(4):546-54.

71. Potts JT, Gardella TJ. Progress, paradox, and potential: parathyroid hormone research over five decades. Ann. N. Y. Acad. Sci. 2007;1117:196-208.

72. Marques MR, da Silva MA, Manzi FR, Cesar-Neto JB, Nociti FH, Jr., Barros SP. Effect of intermittent PTH administration in the periodontitis-associated bone loss in ovariectomized rats. Arch. Oral Biol. 2005;50(4):421-9.

73. Lossdörfer S, Yildiz F, Götz W, Kheralla Y, Jäger A. Anabolic effect of intermittent PTH(1-34) on the local microenvironment during the late phase of periodontal repair in a rat model of tooth root resorption. Clin. Oral Investig. 2010;14(1):89-98.

74. Vasconcelos DF, Marques MR, Benatti BB, Barros SP, Nociti FH, Jr., Novaes PD. Intermittent parathyroid hormone administration improves periodontal healing in rats. J. Periodontol. 2014;85(5):721-8.

75. Makrygiannakis MA, Kaklamanos EG, Athanasiou AE. Effects of systemic medication on root resorption associated with orthodontic tooth movement: a systematic review of animal studies. Eur. J. Orthod. 2019;41(4):346-59.

76. Gonzales C, Hotokezaka H, Matsuo K-I, Shibazaki T, Yozgatian JH, Darendeliler MA, et al. Effects of steroidal and nonsteroidal drugs on tooth movement and root resorption in the rat molar. Angle Orthod. 2009;79(4):715-26.

77. Locklin RM, Khosla S, Turner RT, Riggs BL. Mediators of the biphasic responses of bone to intermittent and continuously administered parathyroid hormone. J. Cell. Biochem. 2003;89(1):180-90.

78. Lossdörfer S, Yildiz F, Götz W, Kheralla Y, Jäger A. Anabolic effect of intermittent PTH (1-34) on the local microenvironment during the late phase of periodontal repair in a rat model of tooth root resorption. Clin. Oral Investig. 2010;14(1):89-98.

79. Bakdach WMM, Hadad R. Effectiveness of low-level laser therapy in accelerating the orthodontic tooth movement: A systematic review and meta-analysis. Dent. Med. Probl. 2020;57(1):73.

80. Altan BA, Sokucu O, Ozkut MM, Inan S. Metrical and histological

investigation of the effects of low-level laser therapy on orthodontic tooth movement. Lasers Med. Sci. 2012;27(1):131-40.

81. Huang H, Williams RC, Kyrkanides S. Accelerated orthodontic tooth movement: molecular mechanisms. Am. J. Orthod. Dentofacial Orthop. 2014;146(5):620-32.

82. Matsuda Y, Motokawa M, Kaku M, Sumi H, Tanne K, Tanimoto K. RANKL and OPG expression: Jiggling force affects root resorption in rats. Angle Orthod. 2017;87(1):41-48.

83. Yang X, Wang Y, Han X, Shu R, Chen T, Zeng H, et al. Effects of TGF-beta1 on OPG/RANKL expression of cementoblasts and osteoblasts are similar without stress but different with mechanical compressive stress. ScientificWorld J. 2015:718180

84. Kim DW, Jo YY, Garagiola U, Choi JY, Kang YJ, Oh JH, et al. Increased Level of Vascular Endothelial Growth Factors by 4-hexylresorcinol is Mediated by Transforming Growth Factor-β1 and Accelerates Capillary Regeneration in the Burns in Diabetic Animals. Int. J. Mol. Sci. 2020;21(10):3473

85. Ren Y, Maltha JC, Kuijpers-Jagtman AM. The rat as a model for orthodontic tooth movement—a critical review and a proposed solution. Eur. J. Orthod. 2004;26(5):483-90.

86. Sameshima GT, Sinclair PM. Predicting and preventing root resorption: Part I. Diagnostic factors. Am. J. Orthod. Dentofacial Orthop. 2001;119(5):505-10.

87. Gonzales C, Hotokezaka H, Yoshimatsu M, Yozgatian JH, Darendeliler MA, Yoshida N. Force magnitude and duration effects on amount of tooth movement and root resorption in the rat molar. Angle Orthod. 2008;78(3):502-9.

88. Sato T, Iida J, S K. A histological investigation on the periodontal tissue changes during molar depression in rats. J. Jpn. Orthod. Soc. 1984;43:361-72.

89. Iglesias-Linares A, Hartsfield Jr J. Cellular and molecular pathways leading to external root resorption. J. Dent. Res. 2017;96(2):145-52.

90. Kim SG. Immunomodulation for maxillofacial reconstructive surgery. Maxillofac. Plast. Reconstr. Surg. 2020;42(1):1-9.

91. Iyengar R, Bohmont CW, Mcevily AJ. 4-Hexylresorcinol and prevention of shrimp blackspot: residual analyses. J. Food Compost. Anal. 1991;4(2):148-57.

92. Amadasi A, Mozzarelli A, Meda C, Maggi A, Cozzini P. Identification of xenoestrogens in food additives by an integrated in silico and in vitro approach. Chem. Res. Toxicol. 2009;22(1):52-63.

93. Kang YJ, Oh JH, Seok H, Jo YY, Kim DW, Garagiola U, et al. 4-Hexylresorcinol exhibits different characteristics to estrogen. Appl. Sci. 2020;10(5):1737.

Table 1. Intergroup comparison of percentage root volume/ total volume.

Group	Male	Female
Control group	69.25 ± 1.51%	68.68 ± 0.79%
Experimental group	71.69 ± 0.88%*	71.52 ± 1.85%*

*$p < 0.05$, comparison between the experimental group and control group. The values are presented as mean ± SD.

Table 2. Intergroup comparison of the amount of tooth movement of the mandibular first molar.

Group	Male	Female
Control group	1.02±0.44 mm	1.14±0.84 mm
Experimental group	0.77±0.11 mm	1.03±0.31 mm

There were no statistically significant differences in both males and females. The values are presented as mean ± SD.

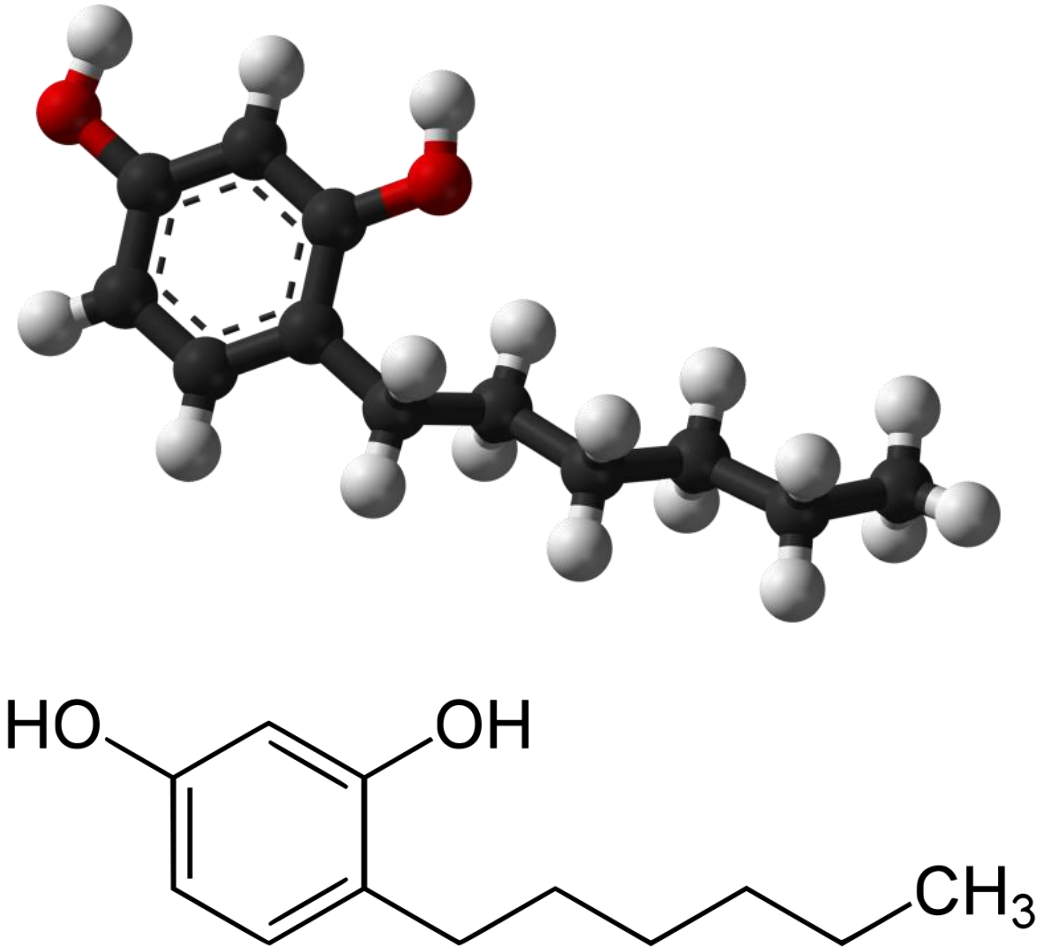


Figure 1. Molecular structure of 4HR(The 3D ball and stick model and molecular formula of 4HR).

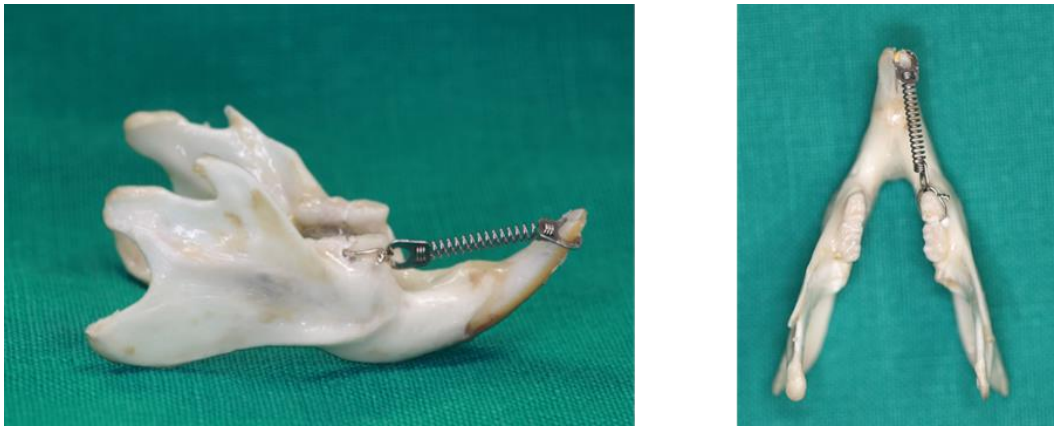


Figure 2. Application of 8 mm nickel-titanium closed coil spring between the right mandibular first molars and incisor of the rat.

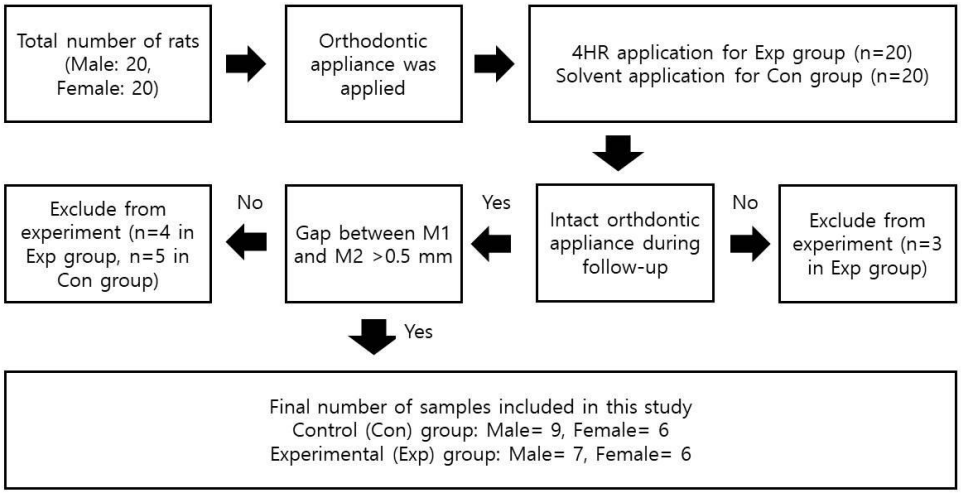
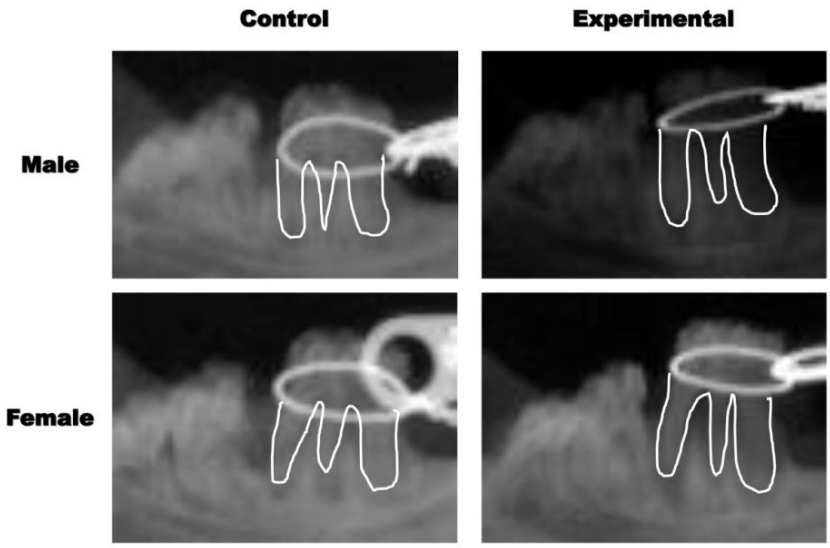
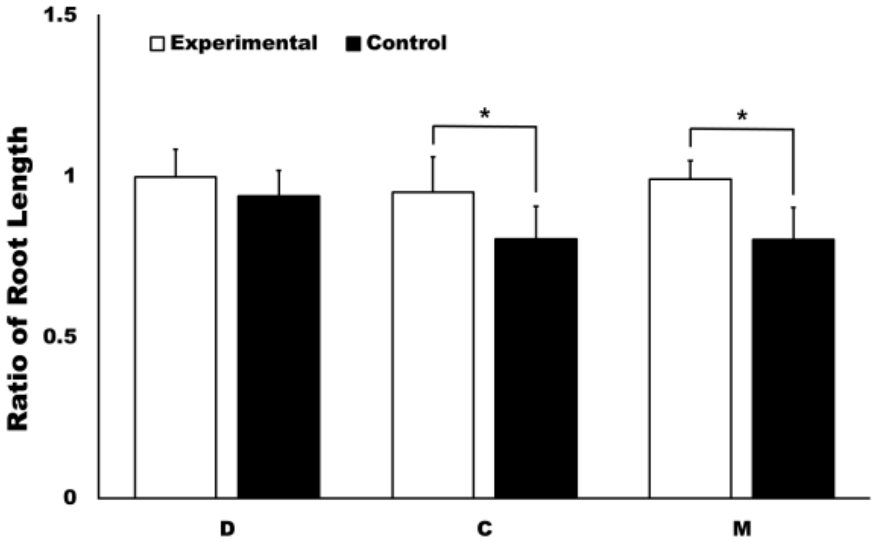


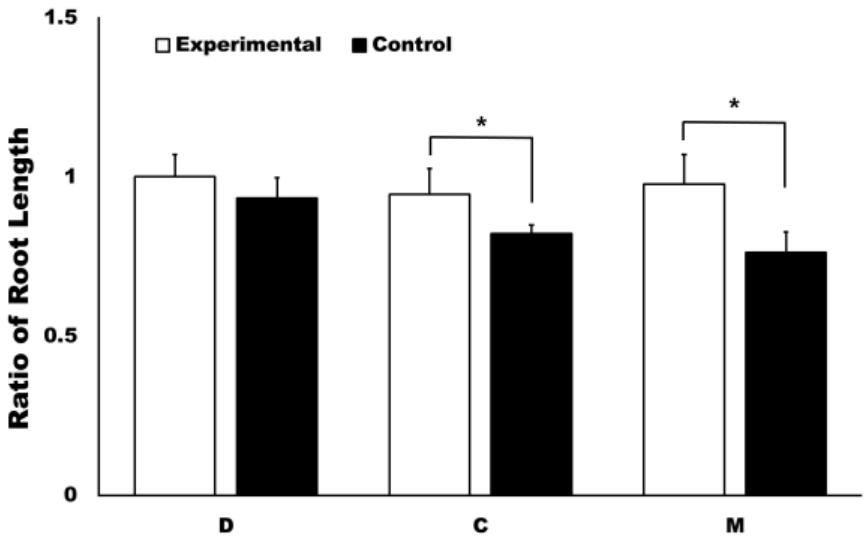
Figure 3. The flowchart for the experimental design.


Control
Experimental
Male
Female

(a)


Experimental
Control
Ratio of Root Length
1.5
1
0.5
0
*
*
D
C
M

(b)



(c)

Figure 4. The ratio of root length. The ratio was calculated between the root lengths of the moved tooth and those of the opposite arch.

(a) Traced outline of roots. The ratio of root length was calculated by comparison with the mandibular first molar in the opposite arch.

(b) The root length comparison in the male group. There was no significant difference in the length of the distal root (D) ($p > 0.05$). However, there was a significant difference in the central roots (C) and the mesial root (M) ($*p < 0.05$).

(c) The root length comparison in the female group. The trend was in accordance with the male group comparison ($*p < 0.05$).

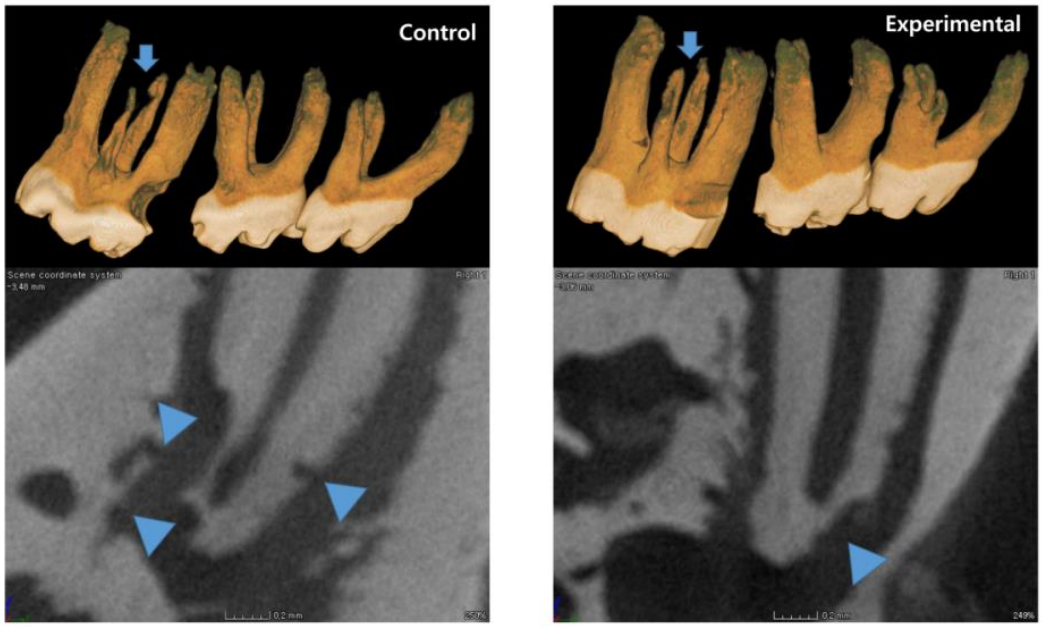


Figure 5. The results of micro-CT analysis. Compared to that in the experimental group, roots resorption was more prominent in the control group. Particularly, central roots (arrow) showed extensive resorption in the control group. In a higher magnification view of the mesial root, both groups showed external root resorptions (arrow heads). Interestingly, lacunas caused by root resorption were found not only on the compression side, but also on the tension side.

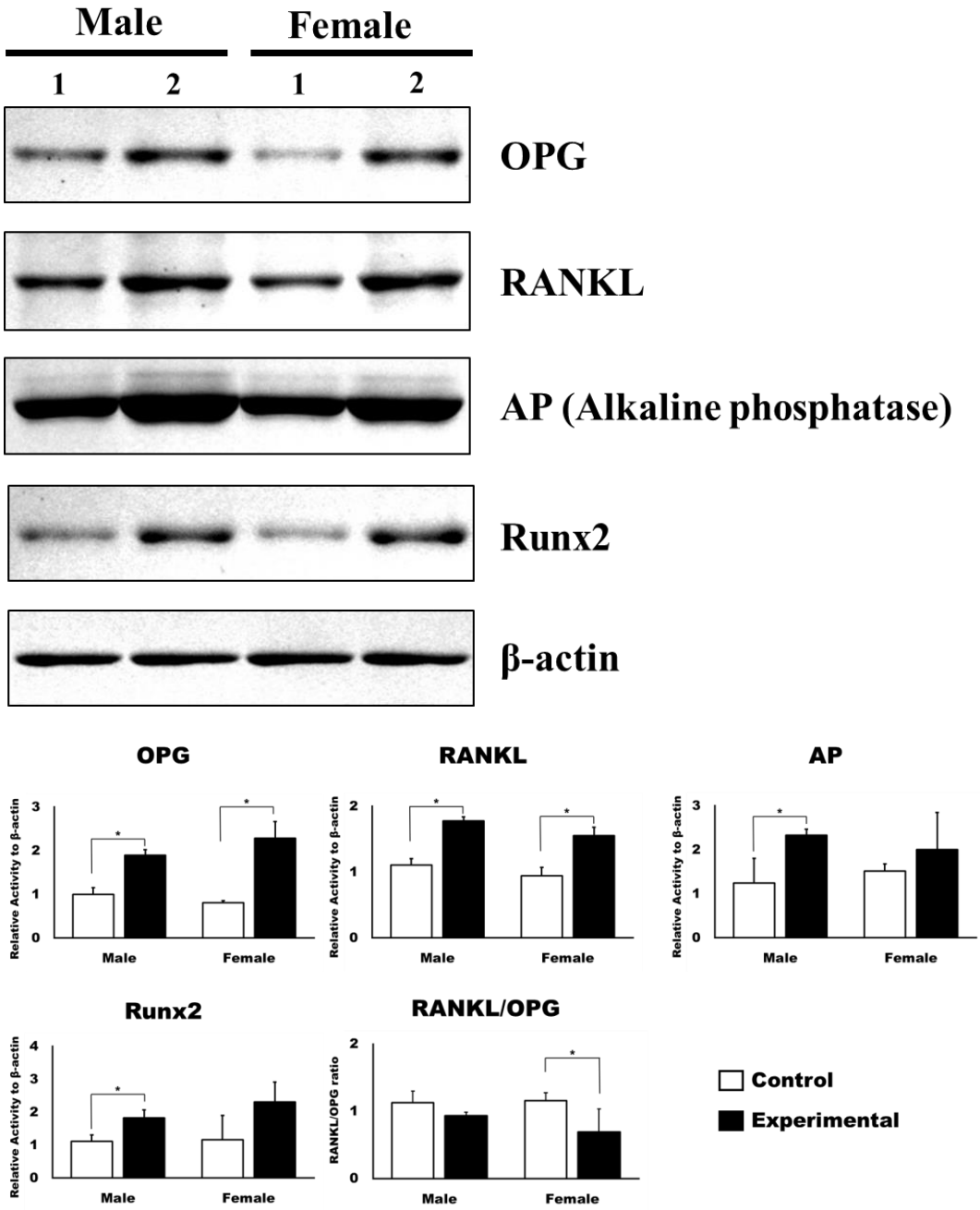

Male
Female
1
2
1
2
OPG
RANKL
AP (Alkaline phosphatase)
Runx2
β-actin
OPG
RANKL
AP
Relative Activity to β-actin
Male
Female
Runx2
RANKL/OPG
RANKL/OPG ratio
Control
Experimental

Figure 6. The results of western blot for tissue samples.

(a) The representative blot images after 4HR administration. Compared to that in the control group, the administration of 4HR increased the expression of OPG, RANKL, AP and Runx−2 (1: control, 2: experimental group).

(b) Relative level of expression of β−actin for each protein was calculated. In addition, the RANKL−to−OPG ratio was calculated. When compared to the control group, the experimental group showed a significantly lower level of expression ($*p < 0.05$).

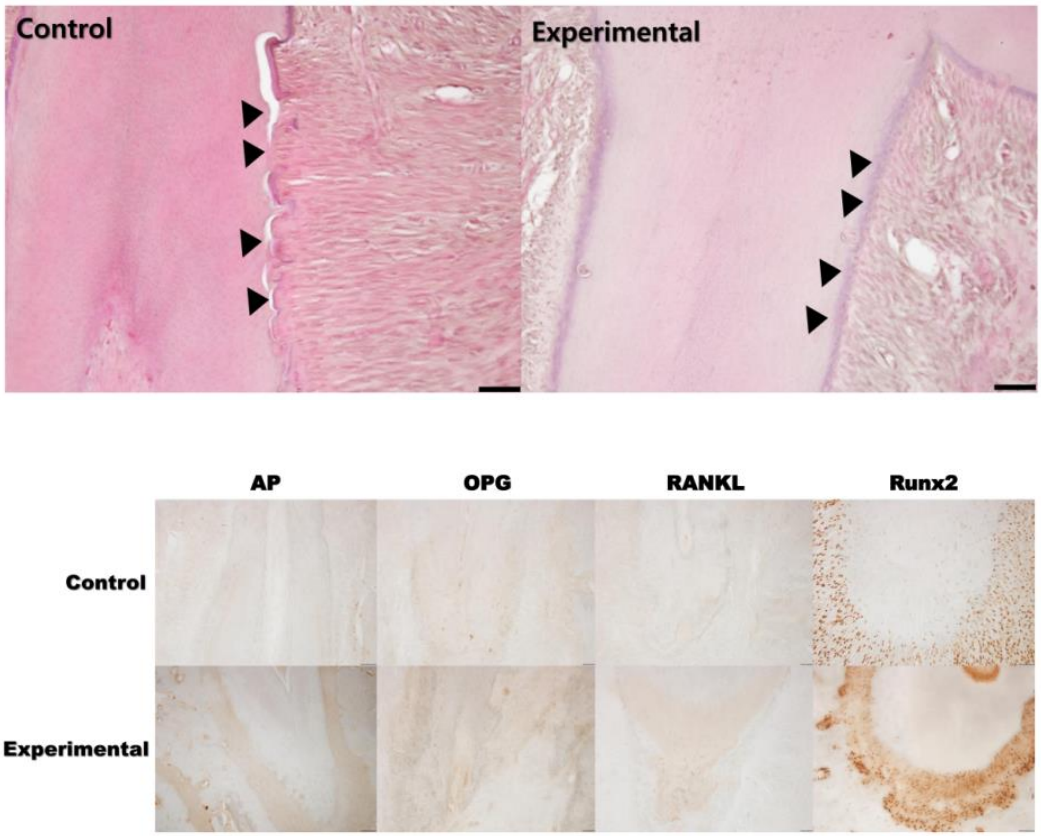


Figure 7. The results of histological analysis.

(a) The control group showed multiple root resorptions (arrow heads). Thin cementum is shown in a violet color, and it was discontinuous because of root resorption. The experimental group showed continuous cementum (arrow heads, hematoxylin and eosin stain, bar= 50 μm).

(b) The immunohistochemical staining results demonstrated that positive staining for AP, OPG, RANKL, and Runx2 was mainly found in the periodontal ligament area. The staining intensity for each marker was

stronger in the experimental group compared to that in the control group (original magnification x 200).

국문 초록

쥐에서 과도한 교정력 부여 시 4-hexylresorcinol의 치근흡수 억제효과에 관한 연구

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목적: 4-Hexylresorcinol (4HR)의 투여는 골 내 리모델링과 골 형성과 관련된 인자를 증가시킨다. 치조골 리모델링 또는 골 전환(bone turnover)은 교정적 치아이동 및 치근흡수에 영향을 미친다. 본 연구는 4HR 이 과도한 교정력을 부여한 후 발생되는 치근흡수에 미치는 영향에 대해 방사선학적 및 조직면역화학적 분석을 통해 알아보고자 한다.

방법: 총 40 마리의 흰 쥐(수컷 20 마리, 암컷 20 마리)에 나이타이 코일 스프링을 이용하여 치근흡수가 일어날 수 있을 만큼 큰 교정력(120g)을 하악 제 1 대구치에 가하였다. 실험군(n = 20)은 2 주마다 12.8 mg/kg 의 4HR 을 투여하였다. 대조군(n = 20)은 4HR 없이 용매만 투여하였다. 교정적 치아 이동 4 주 후 모든 동물을 치근단 방사선 사진 촬영 및 마이크로 컴퓨터 단층 촬영 분석,

웨스턴 블롯 분석 및 면역조직화학분석을 시행하여 치근흡수의 정도를 확인하고 골 전환 인자의 발현 정도를 파악하였다.

결과: 치근단 사진에서 교정력을 가한 대구치와 반대측 대구치의 치근 길이를 측정하였을 때 4HR 투여군에서 대조군에 비해 치근길이의 비율이 컸으며, 마이크로 컴퓨터 단층 촬영 분석으로 전체 치아부피에 대한 치근의 부피 비율을 비교하였을 때도 실험군에서 비율이 더 큰 것을 확인할 수 있었다($p < 0.05$). 웨스턴 블롯과 면역화학조직학적 분석에서 OPG, RANKL, alkaline phosphatase, Runx2 의 발현 수준이 모두 대조군에 비해 실험군에서 유의하게 증가되었다($p < 0.05$). 이들의 발현은 주로 치주인대 근처에서 나타났다. RANKL 과 OPG 모두 발현이 증가되었지만 RANKL/OPG 비율이 실험군에서 더 적음을 확인하였다.

결론: 4HR 투여는 과도한 교정력 하에서 치근흡수를 감소시키고 OPG, RANKL, AP 및 Runx2 의 발현 수준을 증가시켰다.

주요어: 4-hexylresorcinol; 치근흡수; RANKL; OPG; 골리모델링

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