Effect of Nicotine on Orthodontic Tooth Movement and Bone Remodeling in Rats

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Effect of Nicotine on Orthodontic Tooth Movement and Bone Remodeling in Rats

(Directed by Professor Chung-Ju Hwang, D.D.S., M.S., Ph.D.)

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2021 년 8 월 저자 씀

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ABSTRACT

Effect of Nicotine on Orthodontic Tooth Movement and Bone Remodeling in Rats

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Orthodontic tooth movement is mediated by coupling bone resorption and deposition on the compression and tension sides of the periodontal ligament, respectively. Nicotine was found to have a negative effect on bone remodeling via an increase in cytokines involved in bone resorption and oxidative stress which induce osteoclastogenesis and inhibit osteoblastic differentiation. Nicotine itself has been observed to cause dose-dependent bone loss in furcation areas, even in rats with a healthy periodontium, and showed adverse effects on the dynamic histomorphometric parameters of the trabecular bone in a time-dependent manner. Few studies have assessed the effect of nicotine on bone remodeling in a time- and dose-dependent manner in accordance with the stages of orthodontic tooth movement. Thus, the aim of this study was to quantitatively

analyze the effect of nicotine on orthodontic tooth movement and bone remodeling in rats using micro-computed tomography (micro-CT) and tartrate-resistant acid phosphatase (TRAP) immunostaining. We hypothesized that nicotine would deteriorate bone remodeling around teeth with optimal orthodontic forces and result in adverse consequences of orthodontic tooth movement.

Thirty-nine adult male Sprague-Dawley rats were randomized into three groups: group A, 0.5 mL normal saline (n=9, 3 per 3, 7, and 14 days); group B, 0.83 mg/kg nicotine (n=15, 5 per 3, 7, and 14 days); and group C, 1.67 mg/kg nicotine (n=15, 5 per 3, 7, and 14 days). Each animal received daily intraperitoneal injections of nicotine/saline from the day of insertion of identical 30-g orthodontic force delivery systems. A 5-mm nickel-titanium closed-coil spring was applied between the left maxillary first molar (M1) and the two splinted incisors.

The rate of orthodontic tooth movement and volumetric bone changes were measured using micro-CT. Osteoclasts were counted on the mesial alveolar bone surface of the distobuccal root of M1. Six dependent outcome variables, including the intermolar distance, bone volume fraction, bone mineral density, trabecular thickness, trabecular volume, and osteoclast number, were summarized using simple descriptive statistics. Nonparametric Kruskal-Wallis tests were used to evaluate differences among groups at 3, 7, and 14 days of orthodontic tooth movement. Spearman's correlation analyses were conducted to investigate the relationship of the dependent outcome variables with the orthodontic tooth movement rate in the three groups at 3, 7, 14 days.

The results of this study were as follows :

1. The intermolar distance (M1-M2, mm) showed no statistically significant difference among groups at 3, 7, and 14 days. However, the maximum distance was observed on day 14 in group C, while the least distance was observed on day 3 in group A. According to the descriptive statistics, the orthodontic tooth movement rate in the control group gradually increased at regular intervals over time. Compared with the

control group, the nicotine groups showed a fluctuating orthodontic tooth movement rate over the observation period .

2. The bone volume fraction, bone mineral density, trabecular thickness, and trabecular volume showed no statistically significant differences among groups at 3, 7, and 14 days. However, the lowest values for the bone volume fraction, bone mineral density, and trabecular thickness were observed on day 14 in group C. Spearman's correlation analyses showed a significant relationship (correlation coefficient $+1$, $p < 0.01$) between M1-M2 and the bone volume fraction, bone mineral density, and trabecular thickness on day 7 in group A and day 14 in group C.

3. The number of TRAP-positive osteoclasts showed no statistically significant differences among groups at 3, 7, and 14 days. On day 14 in group C, the number of osteoclasts showed a tendency to increase; this coincided with the maximum intermolar distance observed among the three groups and three time points.

The findings of this study suggest that nicotine does not affect orthodontic tooth movement and bone remodeling, although fluctuations according to the stage of orthodontic tooth movement in the nicotine groups should be clearly elucidated in further prospective studies. Currently, it is necessary to accumulate more profound scientific evidence and knowledge regarding the effects of nicotine on orthodontic tooth movement and bone remodeling. Based on available evidence, orthodontists should explain critical guidelines and precautions to current smokers before the initiation of any orthodontic treatment.

Key words : bone remodeling, micro-computed tomography analyses, nicotine, orthodontic tooth movement, osteoclasts

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I. INTRODUCTION

Tobacco smoking is well known the main risk factor associated with chronic destructive periodontal disease which was characteristic of excessive destruction of the supporting periodontal tissues; bone loss, attachment loss, pocket formation, and premature tooth loss. The risk is 5- to 20-fold elevated for a smoker compared to a never-smoker depending on the exposure to smoking (Bergström, 2004).

Nicotine has been reported to enhance constriction of the intact bone vasculature, impair angiogenesis and osteogenesis in areas of ossification such as extraction sockets (Pinto et al., 2002), higher failure rate of dental implant, and compromise wound healing following surgical

therapy (César-Neto et al., 2003). It was also found to have a negative effect on bone remodeling via an increase in cytokines involved in bone resorption (Hapidin et al., 2007), and oxidative stress (Mody et al., 2001; Suda et al., 1993) which induce osteoclastogenesis and inhibit osteoblastic differentiation. Nicotine itself has been observed to cause dose-dependent bone loss in furcation areas, even in rats with a healthy periodontium, and showed adverse effects on the dynamic histomorphometric parameters of the trabecular bone in a time-dependent manner (Bosco et al., 2007; César-Neto et al., 2006; Liu et al., 2010).

Orthodontic tooth movement is mediated by coupling bone resorption and deposition on the compression and tension sides of the periodontal ligament, respectively. Tooth movement by orthodontic force application is characterized by remodeling changes in the dental and paradental tissues, including the dental pulp, periodontal ligament, alveolar bone, and gingiva. These force-induced strains alter vascularity and blood flow in the periodontal ligament, resulting in local synthesis and release of various key molecules such as neurotransmitters, cytokines, growth factors, colony-stimulating factors, and arachidonic acid metabolites. These molecules can affect bone remodeling and the rate of orthodontic tooth movement (Krishnan and Davidovitch, 2006). Many researchers have studied the effects of drugs, hormones and systemic factors (Gameiro et al., 2007), growth factors, cytokines, prostaglandin, alveolar corticotomy causing the regional acceleratory phenomenon (Iino et al., 2007), and low-frequency mechanical vibration (Yadav et al., 2015) on the rate of orthodontic tooth movement and bone metabolism. Sodagar et al. and Bakathir et al. reported that nicotine significantly accelerated the orthodontic tooth movement rate in a dose-dependent manner in rats (Bakathir et al., 2016; Sodagar et al., 2011). However, both study groups measured the orthodontic tooth movement rate by using an interproximal gauge only at 14 days. Moreover, Shintcovsk et al. (Shintcovsk et al., 2014) documented that nicotine affected bone remodeling during orthodontic tooth movement by reducing angiogenesis, osteoclast-like

cells and Howship's lacunae, and delaying the collagen maturation process in the developed bone matrix.

Osteoclasts appear in the periodontal ligament along the alveolar bone surface a few hours after orthodontic force application in rats, which evidenced using positive tartrate-resistant acid phosphatase (TRAP) immunostaining (Tsay et al., 1999; Yadav et al., 2015). In electron microscope studies, ruffled borders of osteoclasts were seen to be in close contact with the resorbing bone surface (Rody et al., 2001). Current quantitative analyses of tooth supporting alveolar bone include histomorphometry, radiography, and micro-computed tomography (micro-CT). Recent studies have shown that micro-CT was more sensitive in measuring tooth supporting bone mass and bone microstructure than conventional methods (Gielkens et al., 2008; Park et al., 2007).

With an increase in life expectancy and awareness of dental health and orthodontic treatment among adolescents, adults, and older adults, there is increased demand for esthetic and more satisfactory outcomes after comprehensive dental treatment using a multidisciplinary approach involving orthodontic treatment. Both patients and orthodontists are aimed at the ideal results of orthodontic treatment continuing for several months or years, which will show optimal teeth alignment in supporting alveolar bone housing, development of biological health of each tooth and surrounding periodontal tissue, dentofacial esthetic advancement, and functional rehabilitation with stable occlusion. Orthodontists should predict the desirable orthodontic tooth movement rate, accompanying bone remodeling, and adaptive paradental tissue changes depending on the orthodontic force applied for mechanotherapy and the modulating factors such as systemic factors, drugs, oral hygiene, and smoking. To our knowledge, few studies have assessed the effect of nicotine on bone remodeling in a time- and dose-dependent manner in accordance with the stages

of orthodontic tooth movement .

Thus, the aim of this study was to quantitatively analyze the effect of nicotine on orthodontic tooth movement and bone remodeling in rats using micro-CT and TRAP-immunostaining. We hypothesized that nicotine would deteriorate bone remodeling around teeth with optimal orthodontic forces and result in adverse consequences of orthodontic tooth movement.

Ⅱ. MATERIALS AND METHODS

1. Experimental animals and study design

The Institutional Animal Care and Use Committee of the University of Yonsei Health System (IACUC) approved this study (the approval number 2018-0252), which conformed to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines. At the start of the experiment, 48 adult (9 weeks of age), male Sprague-Dawley rats (OrientBio, Seoung-Nam, Korea) weighing 300 ± 50 g were kept in plastic cages under a standard 12-hour light/dark photoperiod at a temperature of 21 ± 2°C and humidity of 55%. The rats were fed standard rat food and sufficient water *ad libitum*. The animals were allowed at least a week of acclimatization at the University of Yonsei Health System, and they were weighed at the beginning of the study and every week.

On the basis of the literature, we determined the need for five experimental animals per group at each time point (Eckelman et al., 2007; Liu et al., 2010; Scheibe, 2008). The enrolled rats were randomly divided into three groups: group A, control group, where rats received 0.5 mL normal saline; group B, where rats received 0.83 mg/kg nicotine (NOVO PREMIUM LIQUID 30 mL, natural nicotine 0.95%, NOVO, C&L, Korea) dissolved in normal saline solution; and group C, where rats received 1.67 mg/kg nicotine (NOVO, C&L). The two doses of nicotine, 0.83 mg/kg daily and 1.67 mg/kg daily, were representative of the doses ingested by humans who daily smoke 10 and 20 cigarettes containing 2.0 mg of nicotine each, respectively (Nociti Jr et al., 2001). For insertion of the orthodontic appliance, the rats were anesthetized by intraperitoneal injection of a mixture of 0.9 mg/kg xylazine hydrochloride and 87 mg/kg ketamine hydrochloride (YUHAN, Seoul, Korea) in the left groin area. Every animal received daily saline or nicotine injections from the day of insertion of identical 30-g orthodontic force delivery systems, and evaluations were

performed at 3, 7, and 14 days (Figure 1).

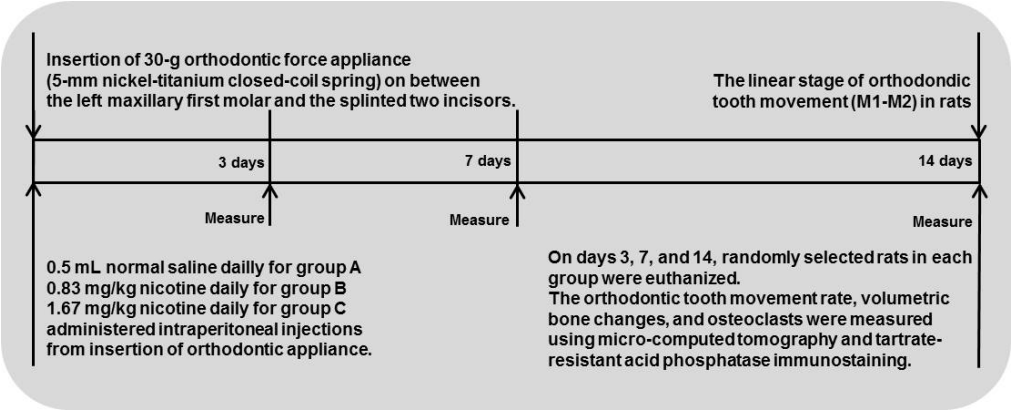

Figure 1. Flowchart showing the design and timeline of the study performed to assess the effects of nicotine on orthodontic tooth movement and bone remodeling in rats.

M1, first molar; M2, second molar

2. Orthodontic force application system

The orthodontic force application system comprised a 5-mm nickel-titanium (Ni-Ti) closed-coil spring (MA-NCC10, 010 × 030; Modern Arch, Wyomissing, PA, USA) that was placed between the maxillary left first molar (M1) and the two splinted incisors and tied with a 0.012-inch stainless steel ligature wire, with delivery of a 30-g mesially directed force to the two anchored incisors (Figure 2). A dial tension gauge (DT-50; Teclock, Nagano, Japan) was used to measure the force and ensure that an identical 30-g force was applied in each animal. Because of the lack of undercuts, the lingual curvature and the eruption pattern of the two maxillary incisors, a sharp,

labial-cervical, edge-shaped groove was prepared using a rotary dental disc (NTI-Kahla Gmbh, Kahla, Germany) in the gingival third of both incisors in order to prevent dislodgment of the ligature wire (Figure 2B). M1 in rats has a slight bulge on the mesiopalatal surface. Accordingly, after passage of the ligature wire through the curvature under the interdental gingiva beneath the contact between M1 and second molar (M2), the wire was threaded under the slight bulge on the mesiopalatal surface of M1. Then, the wire end was buccally turned out in order to relieve discomfort in the palatal area. To reinforce the anterior anchorage, minimize physiological natural distal drifting of the molars, and minimize the continuous eruption of the incisors, the two incisors were joined together to act as a unit (Figure 2C). Self-etching, automix, dual-cured permanent adhesive resin cement (ZIRCONITE™, B.J.M. Laboratories Ltd., Or Yehuda, Israel) was light-cured at both ends of the ligature wire; this secured the Ni-Ti coil spring (Figure 2D). All these steps were repeatedly practiced on a pre-made cast model in order to prepare rat teeth which are 50 times smaller than that of the human molar (Figure 2A). Following insertion of the orthodontic appliance, the rats were allowed to recover with an incandescent light for warmth in their cages. All the animals were fed standard food and sufficient water *ad libitum.*

On days 3, 7, and 14 of orthodontic tooth movement, randomly selected rats from each group were euthanized by carbon dioxide inhalation. The maxilla was enucleated and fixed in 10% neutral paraformaldehyde liquid (Corefix W80; Golden Biotech. Inc., Damyang, Korea) at 4°C for 5 days.

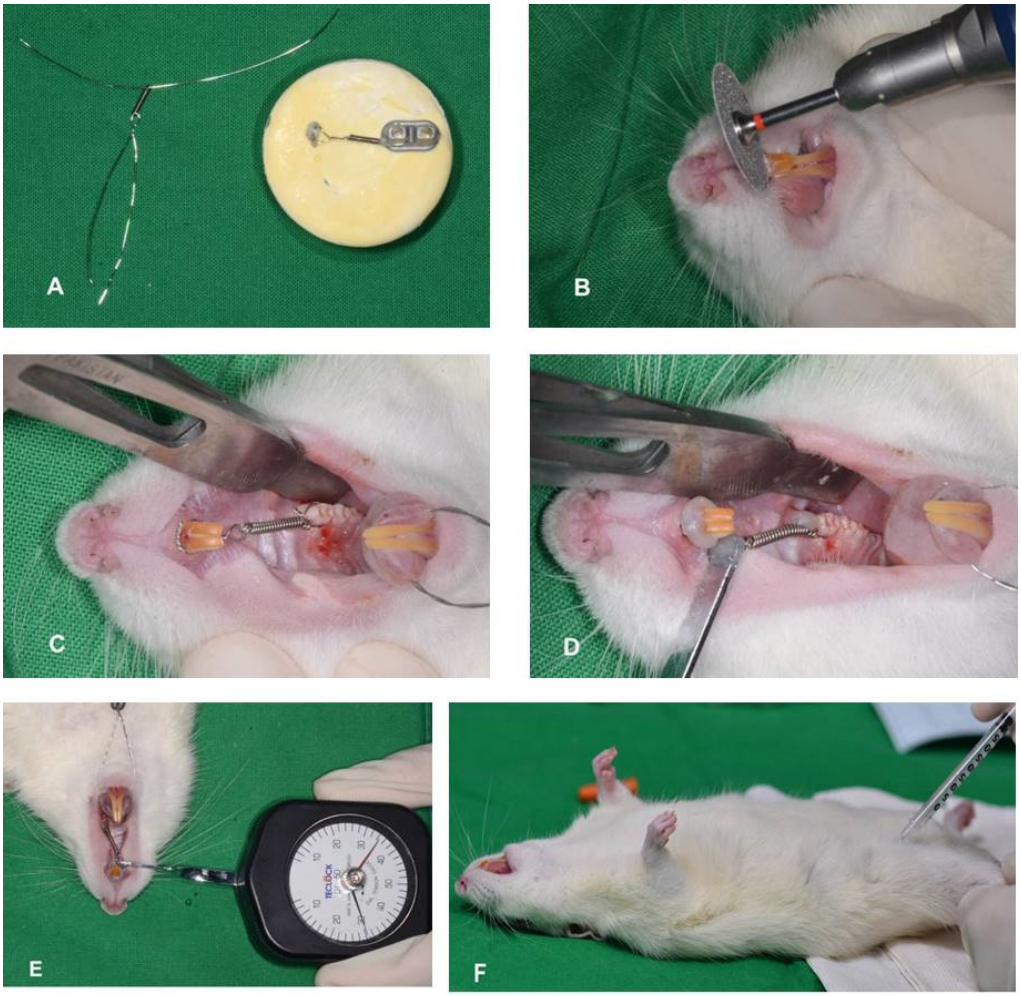

Figure 2. The 30-g orthodontic force application system used to assess the effects of nicotine on orthodontic tooth movement and bone remodeling in rats. **A,** A pre-made cast for image-based training and practice, with a 5-mm nickel-titanium (Ni-Ti) closed-coil spring tied with a 0.012-inch stainless steel ligature wire and a loop for the tension gauge. **B,** Placement of a sharp, labial-cervical, edge-shaped groove in the gingival third of the maxillary central incisors for prevention of ligature displacement. **C,** A 5-mm Ni-Ti closed-coil spring placed between the first molar and

the two incisors, which are splinted for reinforcement of the anterior anchorage. **D,** Self-etching, automix, dual-cured permanent adhesive resin cement was light-cured to both ends of the ligature wire to secure the Ni-Ti coil spring. **E,** Measurement of the 30-g orthodontic force using a dial tension gauge. **F,** Intraperitoneal injection in the left groin area to induce anesthesia for insertion of the orthodontic appliance

3. Micro-CT analyses

For micro-CT (SkyScan 1173 Ver.1.6.0; Bruker, Kontich, Belgium), the X-ray tube source voltage was set to 130 kV, the anode electrical source current was 60 μA, and the image pixel size was 7.10 μm. Three-dimensional (3D) images were reconstructed using the micro-reconstruction Nrecon software (Ver. 1.7.0.4, Bruker).

A. Linear intermolar measurements

Orthodontic tooth movement rate (intermolar distance, M1-M2, mm) was defined as the distance between the most distal point on the M1 crown and the most mesial point on the M2 crown. M1-M2 was measured on two-dimensional (2D) micro-CT sections that were reoriented such that both the cementoenamel junction and the root apex of M1 and M2 appeared in the same slice. Measurements were performed in the sagittal plane, which showed the maximum root structure, at the closest proximity of the two convex molar crown surfaces. M1-M2 in each specimen of each group had been measured on three sagittal micro-CT sections before the orthodontic appliance was untied, which prevented possible errors resulting from relapse (Figure 3). The same operator repeated the measurements three times and calculated the mean value for each animal in order to enhance the intraexaminer reproducibility and reliability.

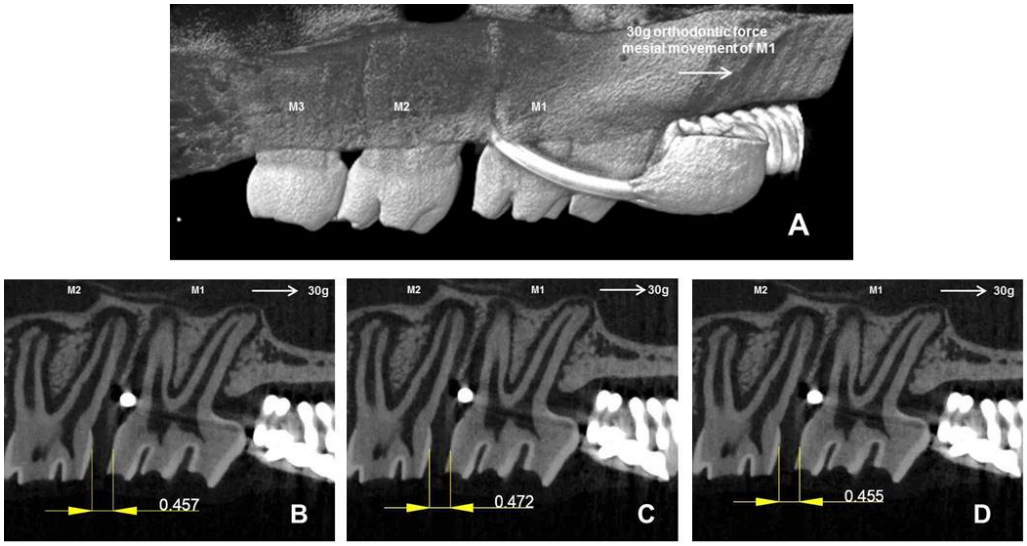

Figure 3. Micro-computed tomography (micro-CT) analyses of the effects of nicotine on orthodontic tooth movement and bone remodeling in rats. **A,** Linear measurements. The intermolar distance (M1-M2, mm) had been measured on three sagittal micro-CT sections for each animal in each group before the the orthodontic appliance was untied, which prevented possible errors resulting from relapse. **B-D,** M1-M2 (mm) was measured on three bidimensional micro-CT sections that were reoriented such that both the cementoenamel junction and the root apex of M1-M2 appeared in the same slice. The same operator repeated the measurements three times and calculated the mean value for each specimen.

M1, first molar; M2, second molar; M3, third molar.

B. Volumetric bone measurements

Quantitative analyses of bone changes occurring in the region of M1 were measured in a 3D region of interest (ROI) (Figure 4), which was defined as follows: vertical, below the roof of the furcation and above the root apex; transverse, the space between the buccal and lingual cortical bone; and sagittal, 100 sections (13 μm) beginning at the mesial root and continuing to the distal root. This 3D ROI was generated by the software based on the resultant 2D contours. The same examiner evaluated all animal specimens and used morphological landmarks when drawing ROIs (CTAn 1.17.7.2+) in order to maximize bone quantification, minimize the inclusion of tooth roots, and use as many reproducible landmarks as possible (Figure 4C). Volumetric bone parameters measured using the established algorithms were as follows: bone volume fraction (bone volume/total volume, %), bone mineral density (apparent bone mineral density in the trabecular bone, g/cm^3), trabecular thickness (maximal spheres in the bone structure, mm), and trabecular volume (mm^3).

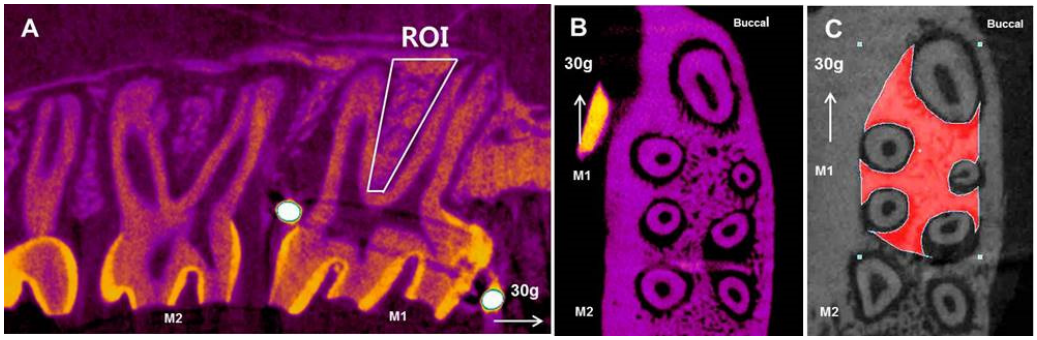

Figure 4. Volumetric measurements obtained by micro-computed tomography to assess the effects of nicotine on orthodontic tooth movement and bone remodeling in rats. **A,** A three-dimensional region of interest (ROI) was marked for quantitative analyses of bone changes occurring in the region of the maxillary first molar. **A, B,** ROI was defined as follows: vertical, below the roof of the furcation and above the root apex; transverse, the space between the buccal and lingual cortical bone; and sagittal, 100 sections (13 μm) beginning at the mesial root and continuing to distal root. **C,** The same examiner evaluated all animal specimens and used morphological landmarks when drawing ROIs (CTAn 1.17.7.2+) in order to maximize bone quantification, minimize the inclusion of tooth roots, and use as many reproducible landmarks as possible

M1, first molar; M2, second molar.

4. TRAP-positive multinucleated osteoclasts

The samples were decalcified in 14% ethylene-diamine-tetraacetic acid for 3 weeks and subsequently processed for standard paraffin embedding. TRAP staining was performed using polymerase chain reaction (PCR)-Cy5 fluorescent gel-based telomerase repeated amplification protocol. TRAP-positive osteoclasts were counted on the mesial alveolar bone surface of the distobuccal root of M1, defined as the compression side during orthodontic tooth movement (Figure 5) (Gonzales et al., 2008; Yadav et al., 2015).

The area for quantification included a square with one side extending from the root apex to the bifurcation and the other side extending 200 μm from the border of the periodontal ligament inside the alveolar bone (Yadav et al., 2015). Digital pathology software (Case-Viewer 2.2.1; 3DHISTECH, Budapest, Hungary) was used on the identically magnifying sectioned image by expressing annotations on each TRAP-positive multinucleated osteoclast (Figure 5B).

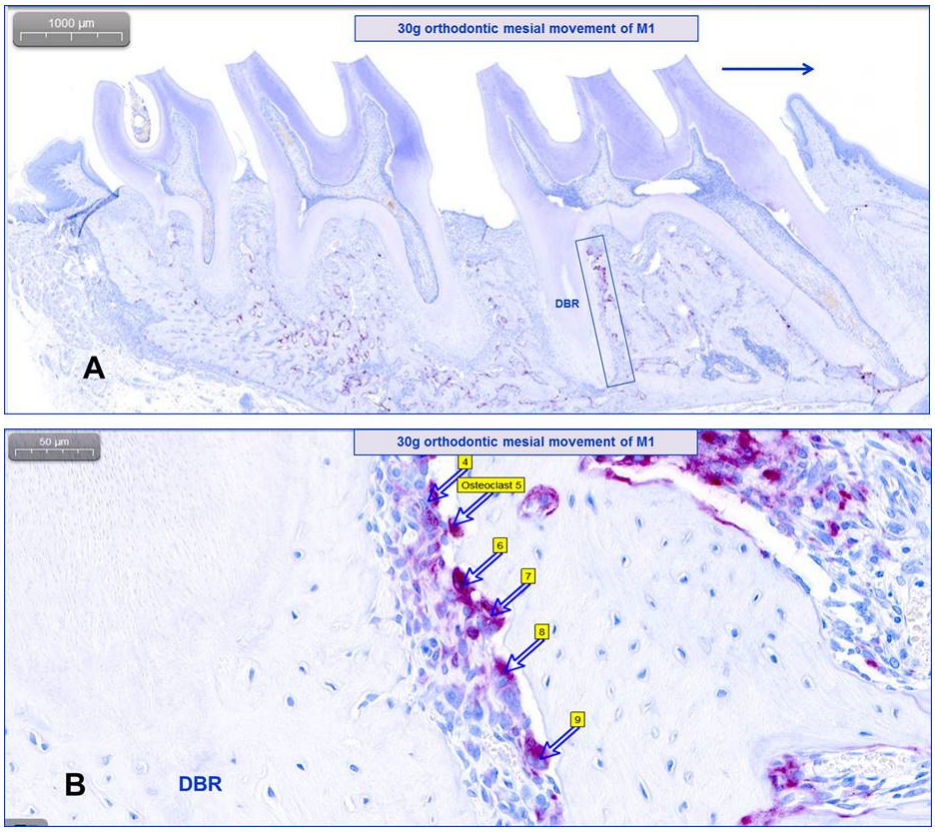

Figure 5. Counting of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated osteoclasts on the mesial alveolar bone surface of the distobuccal root (DBR) of the maxillary left first molar (M1), to assess the effects of nicotine on orthodontic tooth movement and bone remodeling in rats. Digital pathology software was used on identically magnifying sectioned images. **A,** The area for quantification (blue rectangular block on the image) includes a square with one side extending from the root apex to the bifurcation and the other side extending 200 μm from the border of the periodontal ligament inside the alveolar bone of DBR of M1. 1000 μm (original image × 10). **B,** The arrows indicate each TRAP-positive multinucleated osteoclast with expressed annotations by the software. 50 μm (original image × 300).

5. Statistical analysis

Six dependent outcome variables, including the 1) intermolar distance, 2) bone volume fraction, 3) bone mineral density, 4) trabecular thickness, 5) trabecular volume, and 6) osteoclast number, were summarized using simple descriptive statistics. Because of the sample size, the nonparametric Kruskal-Wallis test was performed using SPSS ver. 23.0 (IBM Corp., Armonk, NY, USA) to evaluate statistically significant differences among groups at 3, 7, and 14 days of orthodontic tooth movement (reflecting the dose- and time-dependent effects of nicotine). A *p*-value of < 0.05 was considered statistically significant. In most instances, nonparametric tests are slightly less efficient even when the assumptions of normality, independence within and between samples, identical distribution of each element, and equal variances of the samples are satisfied. On the other hand, they can be significantly more efficient (i.e., require a much smaller sample size) when these assumptions are not satisfied (Eckelman et al., 2007; Scheibe, 2008).

Spearman's correlation analyses were conducted to investigate the relationship of the dependent outcome variables with the orthodontic tooth movement rate in the three groups at 3, 7, 14 days.

Ⅲ. RESULTS

1. Participants

From the 48 animals that were originally fed and prepared for the study, nine were excluded; four could not be awakened from general anesthesia administered for orthodontic appliance insertion; two died after nicotine injection on the fourth and eleventh days of orthodontic tooth movement, respectively; and three were euthanized after dislodgment of the orthodontic appliance. Thus, 39 adult male rats were included in the final analysis. There were nine rats in control group of A (3 per 3, 7, and 14 days) and fifteen rats each in experimental groups of B and C (5 per 3, 7, and 14 days).

2. Linear intermolar measurements

The intermolar distance (M1-M2, mm) showed no statistically significant difference among groups at 3, 7, and 14 days. However, the maximum distance was observed on day 14 in group C, while the least distance was observed on day 3 in group A (Figure 6). According to the descriptive statistics, the orthodontic tooth movement rate in the control group gradually increased at regular intervals over time. Compared with the control group, the nicotine groups showed a fluctuating orthodontic tooth movement rate over the observation period (Figure 7A).

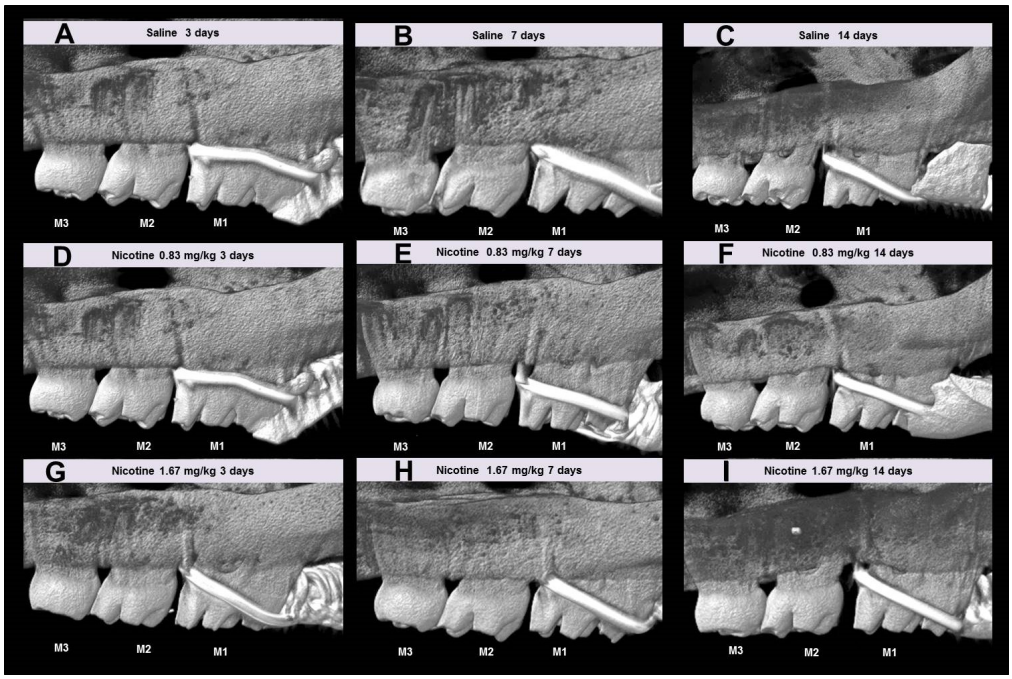

Figure 6. Linear intermolar measurements obtained to assess the effects of nicotine on orthodontic tooth movement and bone remodeling in rats. Representative images for each group showing the intermolar distance (amount of orthodontic tooth movement, M1-M2, mm) on the left side of maxilla at 3, 7, and 14 days after insertion of a 30-g orthodontic force application device (image magnification × 10). **A-C,** Control group (saline 0.5 mL daily). **D-F,** Experimental group (nicotine 0.83 mg/kg daily). **G-I,** Experimental group (nicotine 1.67 mg/kg daily)

M1, first molar; M2, second molar; M3, third molar

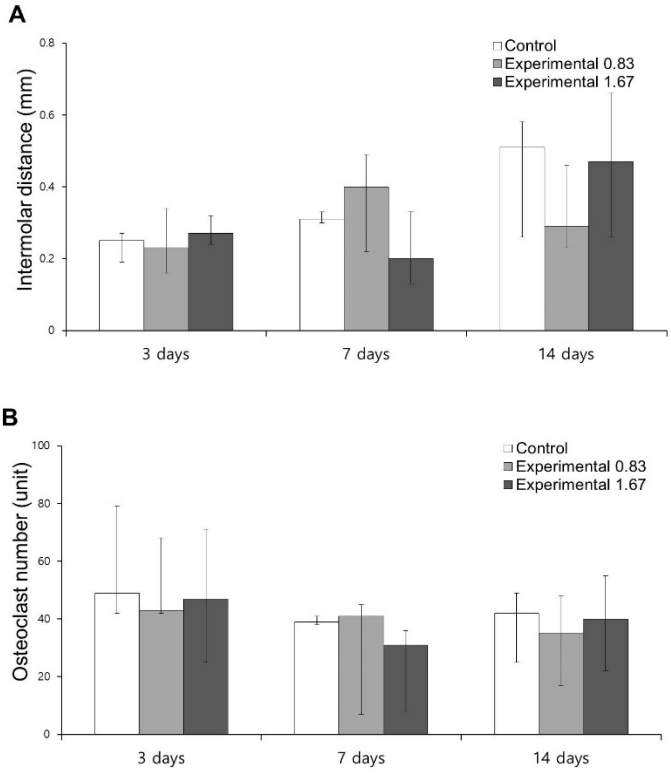

Figure 7. Comparative observations of the intermolar distance and osteoclast number at 3, 7, and 14 days after insertion of a 30-g orthodontic force application device in the control (saline 0.5 mL daily, white bar) and experimental (nicotine 0.83 mg/kg daily, grey bar; nicotine 1.67 mg/kg daily, deep grey bar) groups, to assess the effects of nicotine on orthodontic tooth movement and bone remodeling in rats. **A,** Intermolar distance. **B,** Osteoclast number. The median, maximum, and minimum values at 3, 7, and 14 days were expressed as the representative values for each group.

3. Volumetric bone measurements

The bone volume fraction, bone mineral density, trabecular thickness, and trabecular volume showed no statistically significant differences among groups at 3, 7, and 14 days. However, the lowest values for the bone volume fraction, bone mineral density, and trabecular thickness were observed on day 14 in group C (Figure 8).

Spearman's correlation analyses showed a significant relationship (correlation coefficient $+1$, $p < 0.01$) between M1-M2 and the bone volume fraction, bone mineral density, and trabecular thickness on day 7 in group A and day 14 in group C.

4. TRAP-positive multinucleated osteoclasts

The number of TRAP-positive osteoclasts showed no statistically significant differences among groups at 3, 7, and 14 days (Figure 9). On day 14 in group C, the number of osteoclasts showed a tendency to increase; this coincided with the maximum intermolar distance observed among the three groups and three time points (Figure 7B).

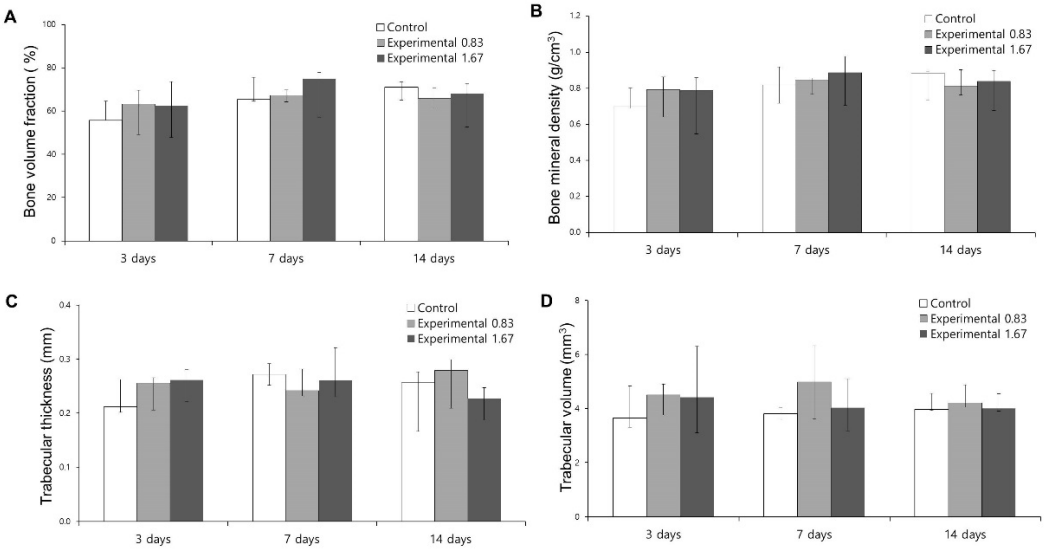

Figure 8. Volumetric measurements of trabecular bone changes occurring in the region of interest in the left maxillary first molar at 3, 7, and 14 days after insertion of a 30-g orthodontic force application device of in the control (saline 0.5 mL daily, white bar) and experimental (nicotine 0.83 mg/kg daily, grey bar; nicotine 1.67 mg/kg daily, deep grey bar) groups, to assess the effects of nicotine on orthodontic tooth movement and bone remodeling in rats. **A,** Bone volume fraction. **B,** Bone mineral density. **C,** Trabecular thickness. **D,** Trabecular volume. The median, maximum, and minimum values at 3, 7, and 14 days were expressed as the representative values for each group.

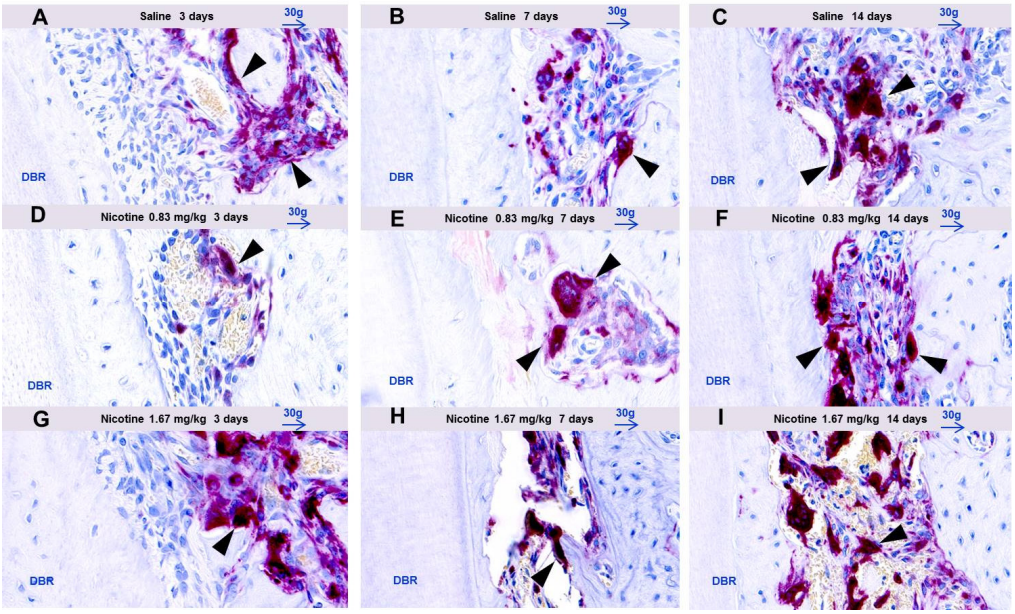

Figure 9. Evaluation of tartrate-resistant acid phosphatase-positive multinucleated osteoclasts (black arrows) on the mesial alveolar bone surface of the distobuccal root (DBR) of the maxillary left first molar at 3, 7, and 14 days after insertion of a 30-g orthodontic force application device to assess the effects of nicotine on orthodontic tooth movement and bone remodeling in rats. Representative images (50-μm, original image × 300) for each group are shown. **A-C,** Control group (saline 0.5 mL daily). **D-F,** Experimental group (nicotine 0.83 mg/kg daily). **G-I,** Experimental group (nicotine 1.67 mg/kg daily)

Ⅳ. DISCUSSION

The alveolar bone in rats is generally more dense than that in humans (Reitan and Kvam, 1971) and tissue changes in rats during orthodontic treatment appear to be faster than in humans, although the principal mechanisms are the same (Brudvik and Rygh, 1993). Despite these differences, rats are generally considered a good model for well-defined, standardized, reproducible force delivery system (Ren et al., 2004). Tissue damage and remodeling in rats start within a few hours, and studies aimed at describing the biological response in the linear phase of tooth movement should have an experimental period of at least 2 weeks (14 days) (Krishnan and Davidovitch, 2006; Reitan and Kvam, 1971). One of the major concerns related to experimental animal studies is the possibility of extrapolating the findings to human clinical settings. According to a recent (2018) systematic review of experimental studies in rats (Michelogiannakis et al., 2018), nicotine exposure in rats jeopardizes orthodontic tooth movement by increasing alveolar bone loss and root resorption. The authors also concluded that further studies need to assess the impact of habitual use of tobacco products on orthodontic tooth movement from a clinical perspective. The present study sought to investigate the effect of nicotine on the initial, lag, and post-lag stages of orthodontic tooth movement and determine the stage at which the consequences of nicotine exposure and orthodontically-induced inflammation were observed.

Shintcovsk et al. (Shintcovsk et al., 2014) showed significant differences in the effect of nicotine on bone remodeling at 3, 7, and 14 days of orthodontic tooth movement in rats. Nociti Jr et al. (Nociti Jr et al., 2001) revealed that daily administration of nicotine 0.37 mg/kg, 0.57 mg/kg, or 0.73 mg/kg could produce direct deleterious effects on the periodontal tissues in rats, including

those without periodontitis. The direct negative effects of the two doses of nicotine 0.83 mg/kg daily and 1.67 mg/kg daily selected in the present study, and orthodontically-induced inflammation could start within a few hours in rats (Nociti Jr et al., 2001). Liu et al. performed micro-CT analysis and showed that nicotine injection after ligation (=induction of periodontitis) for 14 and 28 days increased further alveolar bone loss and decreased the bone mineral density, bone volume fraction, and trabecular thickness in a dose-dependent manner ($p < 0.05$). They also found that in the absence of periodontitis, nicotine itself caused alveolar bone loss in a dose-dependent manner (Liu et al., 2010).

In the present study, 5-mm Ni-Ti closed-coil springs were used to produce a uniform and continuous 30-g orthodontic force during orthodontic tooth movement (King et al., 1991; Yadav et al., 2015). King et al. demonstrated that effective movement of rat molars required a force of 20 to 40 g, and that the velocity of tooth movement did not increase at a force of > 40 g. In a previous study, intermolar distance between M1 and M2 at the linear stage of orthodontic tooth movement were reported about 0.28 mm/week in 20g force, 0.16 mm/week in 40g, and 0.17 mm/week in 60 g for 14 days (King et al., 1991). Sodagar et al. performed an *in vivo* study and showed that nicotine significantly accelerated the orthodontic tooth movement rate in a dose-dependent manner. Accordingly, they suggested that orthodontists should advise their patients to use nicotine replacement therapies to aid in smoking cessation. However, the authors measured M1-M2 (mm) using an interproximal gauge only at 14 days without any other measurement methods (Sodagar et al., 2011). Bakathir et al. found that nicotine significantly accelerated the orthodontic tooth movement rate in a dose-dependent manner; this resulted from unbalanced bone resorption and apposition around moving teeth in rats, as observed through histological assessments. The authors highlighted multiple complications that smokers might experience during and after orthodontic tooth movement (Bakathir et al., 2016). However, they also measured M1-M2 (mm) using an

interproximal gauge only at 14 days. On the other hand, in the present study, a single examiner measured M1-M2 (mm) at 3, 7, and 14 days in accordance with the stages of orthodontic tooth movement on 2D sagittal micro-CT sections. In agreement with the above-mentioned previous studies (Bakathir et al., 2016; Sodagar et al., 2011), the maximum intermolar distance was observed on day 14 in the group receiving the highest dose of nicotine, although the difference among groups was not statistically significant.

Quantifying tooth supporting alveolar bone is complicated because alveolar bone is non-uniform, porous and close proximity to dental hard structures. Histomorphometry provides high resolution and direct representations of alveolar bone levels but has tissue sample destruction. Radiographic methods are non-destructive but have similarities in attenuation of X-rays with the surrounding dental hard tissues (Gielkens et al., 2008; Park et al., 2007). High-resolution micro-CT analyses measure bone parameters and provide detailed, highly reliable, accurate and reproducible 3D information about alveolar hard tissue changes over time in a precise and noninvasive manner (Bouxsein et al., 2010; Liu et al., 2010). In the present study, the fluctuating hard tissue changes over the observation period in the nicotine groups might be associated with the biphasic effects of nicotine on both osteoblasts and osteoclasts. The effects of nicotine on cell proliferation are biphasic; with toxic, antiproliferative effects at high doses (>1 mmol/L) and stimulatory effects at very low doses (0.01–10 μmol/L) (Henemyre et al., 2003; Walker et al., 2001). Nicotine was non-toxic to osteoclasts at clinically relevant levels and appeared to stimulate osteoclast differentiation and resorption of calcium phosphate, which might explain the increased rapidity of periodontal bone loss (Henemyre et al., 2003). The trabecular bone changes observed in the present study showed no significant among group-differences, although they were similar to the changes observed through micro-CT analyses by Liu et al. (Liu et al., 2010). Moreover, our

Spearman's correlation analyses showed a significant relationship (correlation coefficient $+1$, $p < 0.01$) between M1-M2 and the bone volume fraction, bone mineral density, and trabecular thickness on day 7 in group A and day 14 in group C.

The correlation between the number of osteoclasts and the time- and dose-dependent effects of nicotine during the different stages of orthodontic tooth movement has not been widely investigated, with controversial results published in the literature. In the present study, the number of TRAP-positive osteoclasts tended to increase on day 14 in group C, where the nicotine dose was 1.67 mg/kg; this coincided with the finding of the maximum intermolar distance among the three groups and three time points. Shintcovsk et al. reported that male rats who received 2 mg/kg nicotine showed significantly fewer osteoclast-like cells at 7 and 14 days of orthodontic tooth movement (Shintcovsk et al., 2014). Kirschneck et al. demonstrated that orthodontic force application *in vivo* led to a significant increase in nicotine-induced periodontal bone loss, likewise force application *in vitro* enhanced the differentiation of RAW264.7 precursor cells into osteoclast-like cells. For the *in vivo* study, they only used one dose of nicotine 1.89 mg/kg and a small sample of 14 male rats (Kirschneck et al., 2015). Araujo et al. reported that nicotine decreased the number of osteoclasts when dental movement was not induced (nicotine 1mg/kg without tooth movement group). They calculated the mean number of osteoclasts per square micrometer of all periodontal ligaments in order to avoid the risk of bias related to selection of the compression side of the periodontal ligament and alveolar bone (Araujo et al., 2018). Gonzales et al. measured M1-M2 using digitized lateral cephalograms and observed the largest and deepest root resorption craters on the disto-buccal root which appeared to the compression zone, using 3D images obtained via a laser scanning electron microscope (Gonzales et al., 2008). In the present study, osteoclasts were counted on the mesial alveolar bone surface of the distobuccal root of M1, which was defined as

the compression side of orthodontic tooth movement (Yadav et al., 2015).

In addition, nicotine has been reported to induce cytotoxicity and affect bone remodeling through downregulation of osteoprotegerin expression and upregulation of receptor activator of nuclear factor-kappa B ligand expression in periodontal ligament cells (Chen et al., 2015; Lee et al., 2009). Recently, ferric nitrilotriacetate was found to be an oxidizing agent that affects bone metabolism by suppressing bone growth and increasing cytokines as interleukin (IL-)-1 and IL-6, involved in bone resorption (Norazlina et al., 2010).

Ⅴ. CONCLUSION

As the aim of this study, we quantitatively analyzed the effect of nicotine on orthodontic tooth movement and bone remodeling in rats using micro-computed tomography (micro-CT) and tartrate-resistant acid phosphatase (TRAP) immunostaining in the control 0.5 mL normal saline daily of group A, experimental 0.83 mg/kg nicotine daily of group B and 1.67 mg/kg nicotine daily of group C.

1. The intermolar distance (M1-M2, mm) showed no statistically significant difference among groups at 3, 7, and 14 days. However, the maximum distance was observed on day 14 in group C, while the least distance was observed on day 3 in group A. According to the descriptive statistics, the orthodontic tooth movement rate in the control group gradually increased at regular intervals over time. Compared with the control group, the nicotine groups showed a fluctuating orthodontic tooth movement rate over the observation period .
2. The bone volume fraction, bone mineral density, trabecular thickness, and trabecular volume showed no statistically significant differences among groups at 3, 7, and 14 days. However, the lowest values for the bone volume fraction, bone mineral density, and trabecular thickness were observed on day 14 in group C. Spearman's correlation analyses showed a significant relationship (correlation coefficient $+1$, $p < 0.01$) between M1-M2 and the bone volume fraction, bone mineral density, and trabecular thickness on day 7 in group A and day 14 in group C.
3. The number of TRAP-positive osteoclasts showed no statistically significant differences among groups at 3, 7, and 14 days. On day 14 in group C, the number of

osteoclasts showed a tendency to increase; this coincided with the maximum intermolar distance observed among the three groups and three time points.

The findings of this study suggest that nicotine does not affect orthodontic tooth movement and bone remodeling, although fluctuations according to the stage of orthodontic tooth movement in the nicotine groups should be clearly elucidated in further prospective studies. Currently, it is necessary to accumulate more profound scientific evidence and knowledge regarding the effects of nicotine on orthodontic tooth movement and bone remodeling. Based on available evidence, orthodontists should explain critical guidelines and precautions to current smokers before the initiation of any orthodontic treatment. With implementation of the Plan-Do-Study-Act protocol, future studies with different methods and strategies to overcome the limitations of the present study such as recruitment of more samples, observation over a longer period, and utilization of the advantages of micro-CT without compromises to obtain pharmacokinetic data from potentially fewer animals, will further clarify our findings.

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국문 요약

백서에서 니코틴이 교정적 치아이동과 골재생에 미치는 영향

(지도 : 황충주 교수)

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본 연구의 목적은 니코틴의 주입 용량과 시간에 비례하여 교정적 치아이동 초기 단계-지연 단계-지연후 단계에 따라 골재생에 미치는 영향을 고해상도 전산화 단층촬영 분석 (micro-computed tomography analyses)과 파골세포 면역염색법 (tartrate-resistant acid phosphatase immunostaining)으로 정량화하여 비교 분석해보는 것이었다. 문헌을 바탕으로, 니코틴은 적절한 교정력이 적용된 치아를 둘러싼 치조골 재생 변화에 유해한 영향을 미치고 바람직하지 못한 교정적 치아이동을 초래할 것이다라고 가설을 설정하였다.

악골 성장을 마친 39 마리 수컷 백서 모두 동일한 30 g 교정력을 상악 좌측 제 1 대구치와 고정원이 강화된 두 절치 사이에 나이타이(Ni-Ti) 초탄성 스프링 교정장치를 장착하여 전달하고 임의대로 세 그룹: 그룹 A, 대조군 생리식염수 0.5 mL (9 마리, 3 일, 7 일, 14 일 3 마리씩); 실험군 그룹 B, 니코틴 0.83 mg/kg 와 그룹 C, 니코틴 1.67 mg/kg (그룹별 15 마리, 3 일, 7 일, 14 일 5 마리씩)로 분류하여 교정장치 장착 날부터 생리식염수와 니코틴을 매일 복강내 주사하였다. 교정적 치아이동 3 일째, 7 일째, 14 일째에 각 그룹에서 임의로 선택후 희생한 시편들의 교정적 치아이동 양 (상악 제 1 대구치−제 2 대구치 사이거리, M1-M2, mm)과 골재생 지수들 (bone volume fraction, bone mineral density, trabecular thickness, trabecular volume) 그리고 파골세포 수 (osteoclast number)를 정량화하여 중앙값, 최대값, 최소값으로 단순 기술통계를 내었다. 비모수 Kruskal-Wallis 검정으로 교정적 치아이동 시간 3 일, 7 일, 14 일에따른 니코틴 농도별 그룹간의 차이를 통계 분석하였고 스피어만 상관분석으로 교정적 치아이동 양과 다른 종속 변수들의 연관성을 살펴보았다.

다음과 같은 결과를 얻었다.

1. 선형 교정적 치아이동 양은 교정력 30 g 적용후 교정적 치아이동 시간 3 일, 7 일, 14 일에 니코틴 농도별 그룹간에 통계학적으로 유의한 차이를 보이지 않았다. 그러나, 그룹 C 의 14 일에서 최대값을 보였고, 그룹 A 의 3 일에서 최소값을 보여주었다. 대조군의 교정적 치아이동 양은 교정력 30 g 적용후 교정적 치아이동 시간 3 일, 7 일, 14 일에따라 일정한 간격의 차이를 보이며 점진적으로 증가하였다. 반면에 니코틴 그룹에서는 세 관찰 기간동안 변동하는 교정적 치아이동 양을 보여주었다.

2. 골재생 지수 변화는 교정력 30 g 적용후 교정적 치아이동 시간 3 일, 7 일, 14 일에 니코틴 농도별 그룹간에 통계학적으로 유의한 차이를 보이지 않았다. 그러나, 그룹 C 의 14 일에서 가장 낮은 골 부피분율 (bone volume fraction), 골 밀도 (bone mineral density), 섬유주 두께 (trabecular thickness) 값을 보여주었다. 스피어만 상관 분석에서 그룹 A 의 7 일과 그룹 C 의 14 일에, 교정적 치아이동 양과 골 부피분율, 골 밀도, 섬유주 두께간에 유의한 관계 (상관계수 +1, $p < 0.01$)를 보여주었다.
3. 파골세포 수는 교정력 30 g 적용후 교정적 치아이동 시간 3 일, 7 일, 14 일에 니코틴 농도별 그룹간에 통계학적으로 유의한 차이를 보이지 않았다. 교정적 치아이동 양 최대값을 보인 그룹 C 의 14 일에서 파골세포 수의 증가 추세를 관찰할 수 있었다.

이에 본 연구는 니코틴은 교정적 치아이동과 골재생에 미치는 영향이 미미하다는 결론을 얻었다. 그러나 향후 연구에서는 샘플 수와 관찰 기간, 최소한의 실험 동물 수에서 희생없이 약물학적 영향을 알 수있는 삼차원적 이미징 기술의 장점을 활용하는 등 본 연구의 제한점을 개선하고, 니코틴 그룹에서 교정적 치아이동 단계에따라 변동하는 교정적 치아이동 양과 골재생 지수들을 명확하게 입증하는 노력이 필요할 것으로 보인다. 연구와 과학적 지식을 근거로 흡연자에게 모든 형태의 교정 치료전에 임상적으로 중요한 지침서와 주의 사항을 설명할 수 있을 것이다.

핵심이 되는 말: 고해상도 전산화 단층촬영 분석, 교정적 치아이동 양, 니코틴, 치조골 골재생, 파골세포