Original Article

Importance of the early phase of orthodontic force application in the induction of root resorption

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ABSTRACT

Objective: To investigate the effectiveness of early and short-term administration of lithium (Li) during orthodontic force application (OFA) in preventing orthodontically induced root resorption (OIRR) and verify the importance of the early phase of OFA in the induction of OIRR.

Materials and Methods: Bilateral maxillary first molars of 10-week-old male Wistar rats were moved for 14 days using a closed coil spring inserted between the first molar and the incisor. The rats were randomly grouped into three groups: a group receiving Li for the first 4 days, a group receiving Li daily for 14 days, and a control group receiving a vehicle (saline). Orthodontic tooth movement (OTM) was measured using microcomputed tomography on day 14. The OIRR, osteoclasts, and odontoclasts were evaluated via histological analysis. Immunohistochemical staining for the receptor-activated NF-kB ligand and osteoprotegerin was also performed.

Results: The OTM distance did not differ among the three groups, and the pattern of OTM changed from tipping to bodily movement for both Li groups. Early and short-term administration of Li suppressed OIRR on day 14 as effectively as long-term administration for 14 days. The observed odontoclasts on days 4 and 14 were significantly reduced in both Li groups. Osteoprotegerin expression was significantly increased on day 14 in both groups receiving Li relative to the vehicle group.

Conclusions: Early and short-term Li administration effectively suppressed OIRR. This suggests that the early phase of OFA plays an important role in the induction of OIRR. (*Angle Orthod*. 2025;95:323–331.)

KEY WORDS: Early phase; Root resorption; Lithium; Short term; Odontoclast; Osteoclast

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INTRODUCTION

Orthodontic tooth movement (OTM) is caused by alveolar bone remodeling when orthodontic forces are applied, and it frequently induces orthodontically induced root resorption (OIRR). OIRR is a widespread problem in orthodontics and significantly impacts patient outcomes. Authors of clinical and histological studies have shown that OIRR, including its mild form, is observed in more than 90% of patients who have received orthodontic treatment.[1] Authors of previous studies have shown that higher forces, longer movement distances, and prolonged orthodontic treatment contribute to OIRR.[2–5] OIRR is currently considered among the most challenging problems in orthodontics because it is difficult to predict, and no methods of prevention have been established.[6]

Authors of various clinical and experimental studies have investigated the processes and mechanisms underlying OIRR,[6,7] but no definitive biological mechanisms have been established. Macrophages, osteoclasts, and odontoclasts are widely reported to play roles in the development of OIRR. OIRR is initiated by ischemia-induced cell death (apoptosis) of periodontal ligament (PDL) cells due to the compressive force of orthodontic treatment.[8–10] This is followed by osteoclast and odontoclast differentiation and alterations in alveolar bone metabolism, which ultimately lead to OIRR. However, it remains unknown whether the tissue changes provoking OIRR, from early-phase PDL ischemia to the appearance of odontoclasts in the late phase, are sequential events triggered by PDL compression.

The early phase of PDL compression, occurring around 1 to 4 days postorthodontic force application (post-OFA), has recently been implicated in the induction of OIRR. The PDL was maximally compressed during this early phase, and a strong positive correlation between the early but not the late-phase PDL compression ratio and OIRR induction was demonstrated in rats.[11] These results suggested that early phase PDL ischemia due to PDL compression triggers the induction of OIRR and may determine the degree of OIRR induction. However, no interventional studies have been conducted on the impact of early phase PDL compression on OIRR induction or its processes.

Authors of several studies have investigated medications to control OIRR.[6] Daily administration of lithium (Li) during the experimental period, which is clinically used to treat bipolar disorder, has been shown to suppress OIRR in rats.[12,13] Li was also reported to suppress OIRR through two mechanisms: (1) prevention of ischemia-induced apoptosis of PDL cells during the early phase and (2) suppression of odontoclast differentiation during the late phase.[10]

These findings suggest that the early phase of OFA is important for the induction of OIRR and provide rationale for early and short-term Li administration to prevent OIRR. Therefore, in this study, we aimed to investigate the effect of short-term Li administration restricted to the early phase of OFA on OIRR, along with the biological mechanisms involved in the process for OIRR in rats. The results of this study will confirm the importance of the early phase of OFA in the induction of OIRR and determine whether the tissue changes that provoke OIRR, from early-phase PDL ischemia to the appearance of odontoclasts, are sequential events.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of Nagasaki University Graduate School of Biomedical Sciences (No. 2010011668-4).

OIRR Rat Model

Thirty-two male Wistar rats aged 10 weeks (SLC, Shizuoka, Japan; body weight, 206.9-249.1 g) were used in this study. The rats were housed in plastic cages in a colony room, fed a standard pellet diet and water ad libitum, and acclimatized for at least 72 h before the experiments. A 25 cN nickel-titanium closed-coil spring (Sentalloy, Tommy, Fukushima, Japan) was placed bilaterally between the maxillary first molar (M1) and incisors to induce mesial movement of M1 for 14 days (Figure 1A). A self-curing resin was placed on the surface of the ligature to prevent loosening. All surgeries were performed under general anesthesia with an intraperitoneal injection of 0.375 mg/kg medetomidine (Zenoaq, Fukushima, Japan), 2 mg/kg midazolam (Sandoz, Tokyo, Japan), and 2.5 mg/kg butorphanol tartrate (Meiji Seika Pharma Co. Ltd., Tokyo, Japan).

Administration of Lithium

The rats were randomly divided into three groups: a group receiving lithium chloride (LiCl; Wako, Osaka, Japan) for the first 4 days only (4dLi; $n = 16$), a group receiving LiCl daily for 14 days (14dLi; $n = 16$), and a control group receiving a vehicle daily for 14 days (CNT; $n = 16$). LiCl was dissolved in saline (0.64 mM/kg/day) and administered intraperitoneally. In the 4dLi group, the same volume of vehicle (saline) was administered from days 5 to 14 (Figure 1B).

Microcomputed Tomography Images and Measurement of OTM

Microcomputed tomography (micro-CT; R_mCT, Rigaku, Tokyo, Japan) images were obtained under

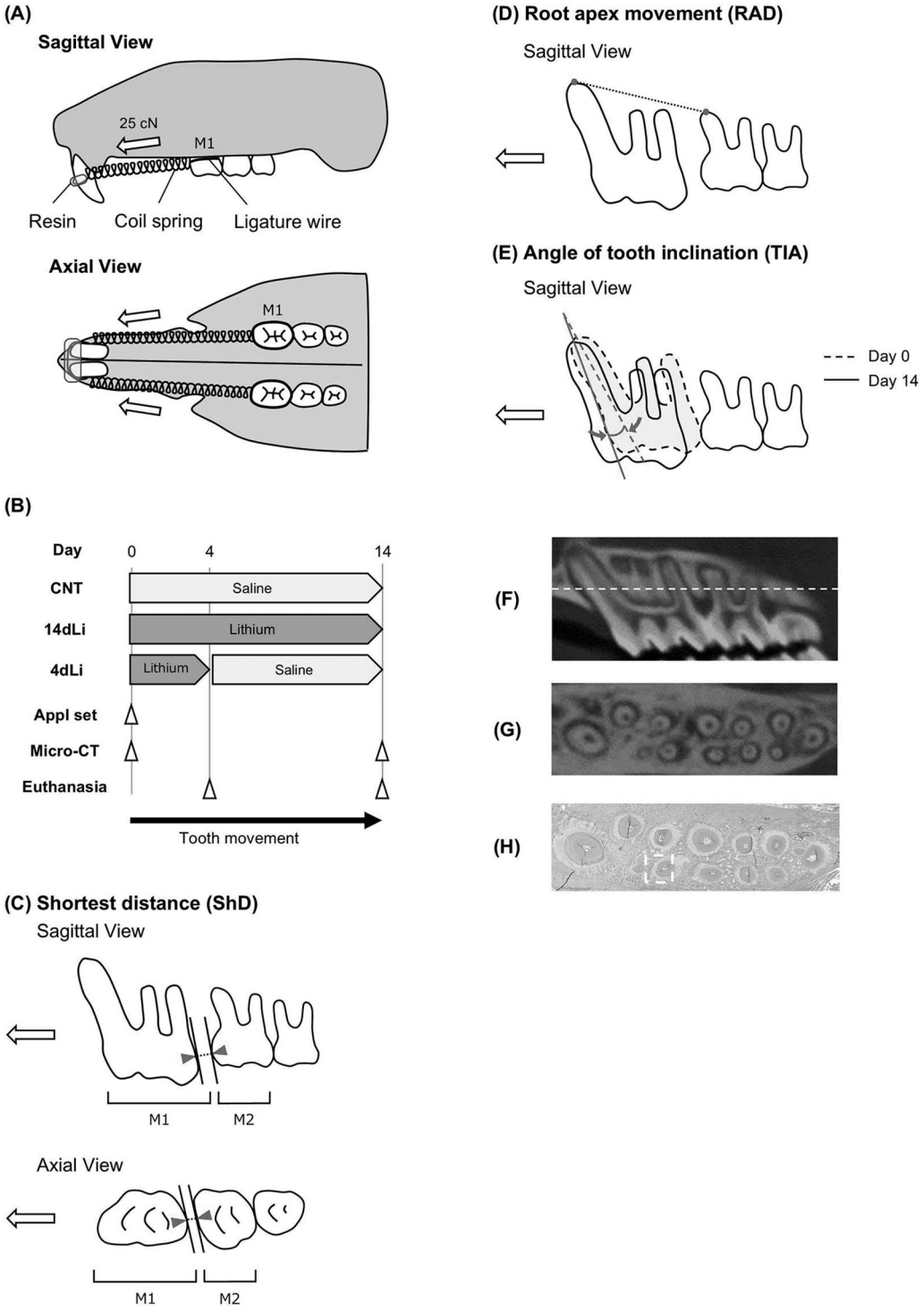

Figure 1. Experimental protocol. (A) Sagittal and axial views of the appliance. The arrows indicate the direction of the orthodontic force. The appliance at the incisors was fixed with composite resin to prevent detachment from the tooth. (B) Experimental schedule. Methods to measure tooth movement: (C) shortest distance, (D) root apex movement, and (E) angle of tooth inclination. (F) Microcomputed tomography (micro-CT) image of the left maxillary molars. The dotted line indicates the position of the sliced tissue section, which is located at the cervical third of the distobuccal root of the maxillary first molar (M1). (G) An axial micro-CT image at the level of the dotted line in (F). (H) Hematoxylin and eosin staining corresponding to the image shown in (G). The white dotted box indicates the measurement area of the distobuccal root of M1. Appl, orthodontic appliance.

anesthesia on days 0 (before the orthodontic appliance was applied) and 14 (Figure 1B). The image acquisition conditions were voltage 90 kV, current 100 μA, exposure time 2 min, and resolution 20 μm/pixel. Three parameters were defined to measure OTM: (1) shortest distance (ShD), the shortest distance between the maxillary M1 and second molar (M2; Figure 1C); (2) root apex movement (RAD), the change in the distance between the root apex of the mesial root of M1 and M2 (Figure 1D); and (3) angle of tooth inclination (TIA), the change in the mesial root inclination of M1 before and after OTM in the sagittal images (Figure 1E).

Preparation for Histological Analysis

Rats in each group were euthanized on days 4 and 14 ($n = 8$ per group). After euthanasia under deep anesthesia, the maxilla was dissected and immersed in a fixative solution of 4% paraformaldehyde in 50 mM sodium cacodylate buffer (pH 7.4) for 48 hours. The maxillary bone tissue was decalcified with 17% ethylenediaminetetraacetic acid (pH 7.4; Osteosoft, Merck Millipore, Darmstadt, Germany) for 4 weeks at room temperature, dehydrated, and embedded in paraffin. Continuous sections (thickness, 6 μm) were prepared at one-third of the cervical region of the distobuccal root of M1 of each specimen (Figures 1F–H).

Hematoxylin-Eosin Staining and Tartrate Resistant Acid Phosphatase Staining

Hematoxylin and eosin staining was used to observe the cross-sectional structures. The OIRR was measured using ImageJ software (National Institutes of Health, Bethesda, MD). Tartrate-resistant acid phosphatase (TRAP) staining was performed to identify and count osteoclasts and odontoclasts. Naphthol AS-MX phosphate (Sigma, St. Louis, MO); N, N-dimethylformamide (Fujifilm Wako assay, Tokyo, Japan); 0.2 M acetate buffer; and distilled water with sodium tartrate dihydrate were stirred and filtered and used for staining for 1 hour. The sections were counterstained with hematoxylin for 1 min.

Immunohistochemical Staining

The tissue sections were deparaffinized in xylene, dehydrated in alcohol, and incubated in 10% citric acid buffer for 30 minutes at 90°C to reconstitute the antigenicity of the protein. After rinsing with phosphate-buffered saline, endogenous peroxidase activity was inactivated by treatment with 0.3% H_2O_2/methanol for 30 minutes at room temperature. Tissue sections were washed and blocked with 10% normal goat serum. They were incubated overnight at 4°C with primary antibodies: mouse monoclonal RANKL antibody (1:100; ab45039 Abcam, Cambridge, England) or rabbit polyclonal anti-OPG antibody (1:100; ab73400 Abcam). Afterward, they were stained using the Histofine SAB-PO (MULTI) kit (Nichirei, Tokyo, Japan), according to the manufacturer's protocol. The visualization of peroxidase activity was performed with 3,3′-diaminobenzidine (Nichirei), followed by rinsing and counterstaining with hematoxylin for 1 minute.

Statistical Analysis

All statistical analyses were performed using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan). One-way analysis of variance and Tukey's post hoc test were used for comparisons. All data are expressed as mean ± standard error. Statistical significance was set at $P < .05$. All measurements were performed in triplicate by the same investigator.

RESULTS

Tooth Movement

Micro-CT scans were used to evaluate OTM after 14 days of orthodontic treatment (Figure 2). The ShD, expressing the OTM distance, was not affected by LiCl administration (Figure 2A). RAD values were higher in both LiCl-treated groups than the CNT group (Figure 2B). TIA significantly decreased after LiCl administration (Figure 2C). Additionally, all three parameters of tooth movement in the 4dLi group were comparable with those in the 14dLi group. These results indicated that the OTM distance was similar in the three groups; however, LiCl treatment changed the OTM pattern from tipping to bodily movement, and this pattern change occurred in both the 4dLi and 14dLi groups.

Histological Evaluation

OIRR was observed on the compression side on day 14 (Figures 3A–C). The area of OIRR was significantly smaller in the two Li groups than in the CNT group ($9.72 \times 10^3 \pm 5.95 \times 10^3$ μm^2 vs $3.60 \times 10^3 \pm 1.17 \times 10^3$ μm^2 vs $4.49 \times 10^3 \pm 1.66 \times 10^3$ μm^2, $P < .01$; Figure 3D). These results demonstrated that OIRR was reduced by the administration of LiCl, and the effects in the 4dLi and 14dLi groups were comparable.

TPAP-positive cells located on the compressive surface of the alveolar bone were defined as osteoclasts, and those located on the compressive side of the root surface were defined as odontoclasts (Figures 4A, B). Osteoclasts were observed on days 4 and 14, and LiCl administration for 14 days significantly reduced them throughout the experimental period ($P < .05$: between the 4dLi and CNT groups

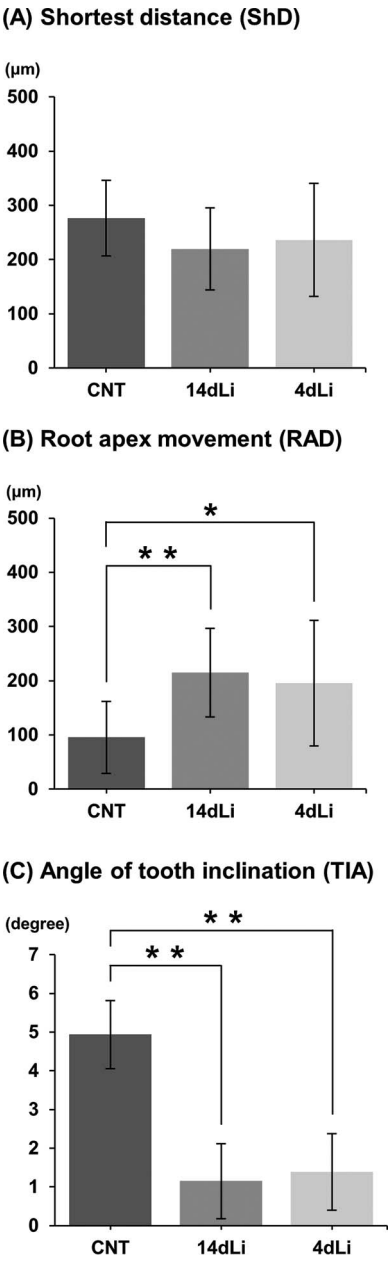

Figure 2. Measurements of tooth movement.

on day 4; $P < .01$: between the 14dLi and CNT groups on day 14; Figure 4C). However, no difference was found between the 4dLi and CNT groups on day 14. The number of odontoclasts appeared to be low on day 4 for the CNT group and increased substantially on day 14. Odontoclasts were significantly reduced by both the 4- and 14-day LiCl treatments ($P < .05$: between 4dLi and CNT groups on day 4; $P < .01$: between 4dLi and CNT groups on day 14 and between 14dLi and CNT groups on day 14; Figure 4D). These results indicated that the appearance of osteoclasts preceded the appearance of odontoclasts, and the appearance of osteoclasts was suppressed in the 14dLi group. In contrast, the appearance of odontoclasts was equally suppressed in both the 4dLi and 14dLi groups.

Both RANKL and OPG were detected in the PDL tissues of the CNT and Li groups (Figures 5A, C). RANKL expression was barely observed on day 4, but it increased on day 14 (Figure 5B). The mean optical density of RANKL was not significantly different for the group on days 4 and 14. In contrast, OPG expression did not change from day 4 to 14 in CNT, but the mean optical density of OPG significantly increased for both Li groups on day 14 ($P < .01$; Figure 5D).

DISCUSSION

In the present study, we show that early short-term administration of Li significantly inhibited OIRR, increased OPG expression, and suppressed odontoclast appearance. These results were comparable with those obtained with long-term Li administration. In addition, Li treatment changed the OTM pattern from tipping to bodily movement, regardless of the 4- or 14-day administration. Thus, early short-term administration of Li effectively suppressed OIRR and had a beneficial effect on OTM in rats. With this study, we are the first to demonstrate the crucial effects of the early phase of OFA on OIRR and OTM.

In the present study, similar reductions of root resorption were observed in the 4dLi and 14dLi groups. The number of odontoclasts was also reduced to the same extent for both Li groups. These findings suggest the following: (1) the first 4 days of Li administration may suppress odontoclast differentiation and OIRR by alleviating or reducing early phase PDL ischemia via OFA-induced compression, and (2) a series of tissue changes provoking OIRR, from early phase PDL ischemia to the appearance of odontoclasts, can be perceived as sequential events triggered by PDL compression. Therefore, the early phase of the OFA may determine the degree of OIRR induction.

RANKL and OPG are involved in the bone remodeling process during orthodontic treatment.[14,15] RANKL is essential for the formation and activation of osteoclasts, while OPG inhibits osteoclast differentiation. In this study, RANKL expression was not altered, but OPG expression was significantly increased in both Li groups relative to that in the CNT group. Authors of a previous study, using low-dose Li supplementation in mice, showed a significant increase in OPG expression, with unaltered RANKL expression.[16] Authors of several in vitro studies have also reported that Li treatment increases OPG.[17,18] This suggests that the suppression of odontoclasts and osteoclasts by Li during

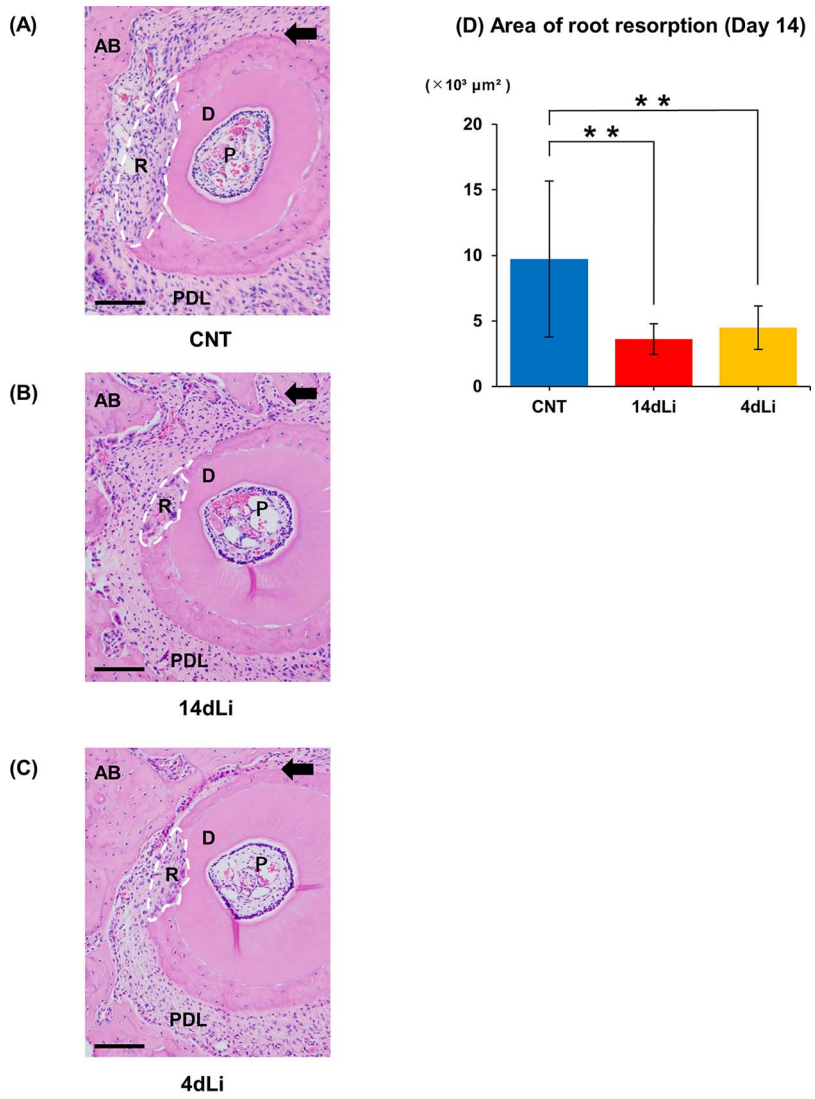

Figure 3. Hematoxylin and eosin staining for the (A) control (CNT), (B) 14-day LiCl (14dLi), and (C) 4-day LiCl (4dLi) groups on day 14 (magnification 20 × 20). The area marked with a white line indicates root resorption (R). (D) The measured area of root resorption. D, dentin; P, pulp; PDL, periodontal ligament; AB, alveolar bone; arrows indicate direction of orthodontic force; scale bars = 100 μm. ** $P < .01$.

OFA is related to the increased expression of OPG and not RANKL.

Li treatment changed the OTM pattern from tipping to bodily movement regardless of the 4- or 14-day administration. These results indicate that the first 4 days of Li administration induced a change in the OTM pattern. This may be attributed to the suppression of osteoclasts on day 4 in the Li group relative to the CNT group. Alternatively, the 4-day Li administration may have caused a difference in bone remodeling between the cervical region and root apices of the alveolar bone. Further studies are needed to elucidate the mechanisms underlying these changes in OTM patterns.

The new findings obtained in this study deepen the understanding of the underlying mechanisms of OIRR and demonstrate the importance of early intervention in its prevention. These insights will be beneficial for clinical practices aimed at reducing the incidence of this adverse event. In fact, Li has been used clinically, and its effects and side effects are well recognized. Therefore, Li can be used to prevent OIRR through early short-term administration as a prophylactic agent, especially in patients at a high risk of OIRR. With short-term administration, fewer concerns regarding side effects exist. However, despite their importance, the results cannot be directly applied in clinical settings at this point, as Li administration for the prevention of OIRR is still in the experimental stage. Therefore, further studies are required to determine the optimal dosage and timing of Li administration in orthodontics. Additionally, investigating long-term effects on OIRR and orthodontic outcomes would provide insight into the sustainability and safety of this intervention.

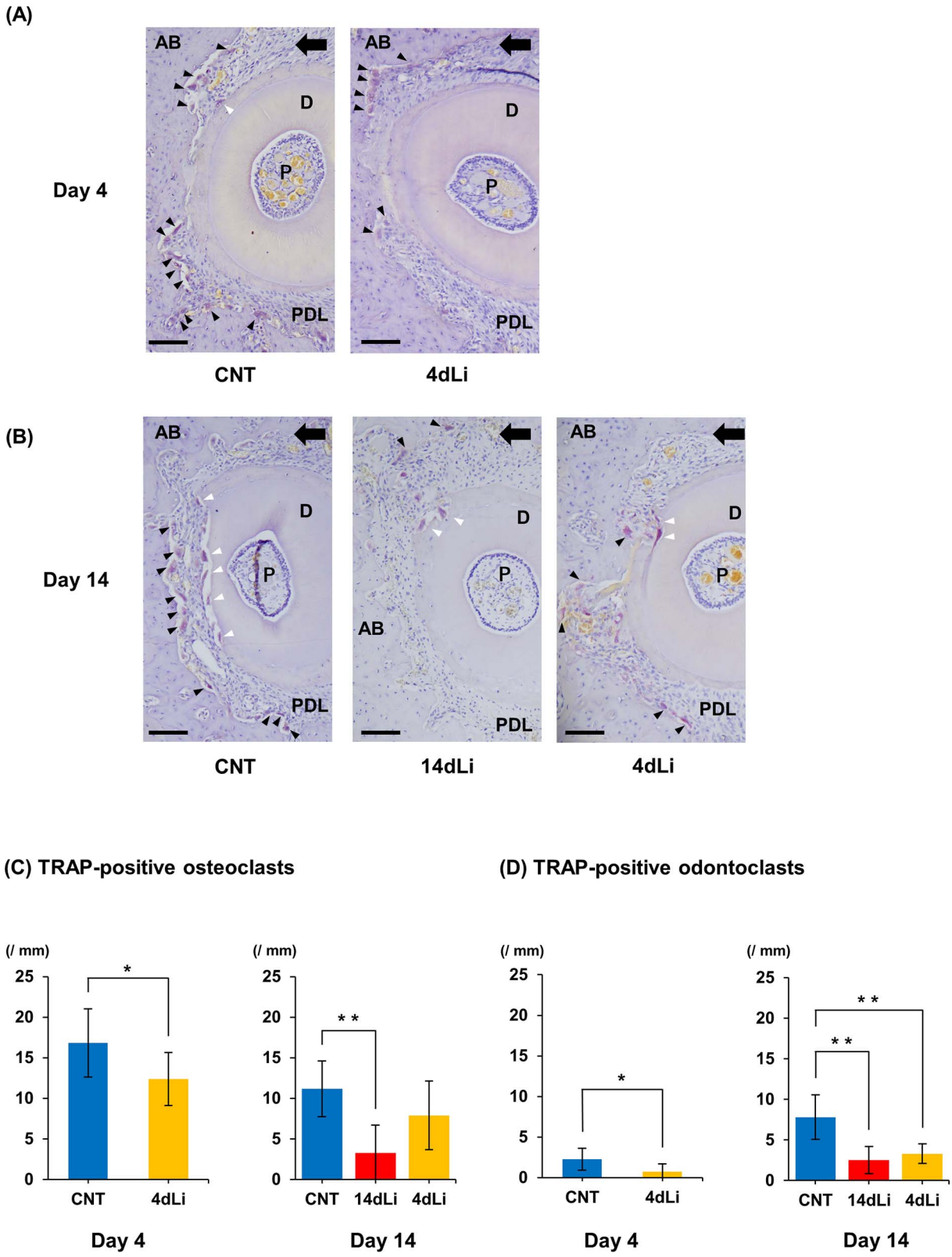

Figure 4. (A) Tartrate-resistant acid phosphatase (TRAP) staining for the control (CNT) and 4-day LiCl (4dLi) groups on day 4 (magnification 20 × 20). Osteoclasts and odontoclasts are stained in red. Black arrowheads indicate osteoclasts and white arrowheads indicate odontoclasts. (B) TRAP staining in the CNT, 14-day LiCl (14dLi), and 4dLi groups on day 14 (magnification 20 × 20). (C) The number of TRAP-positive osteoclasts in the CNT, 14dLi, and 4dLi groups on days 4 and 14. (D) The number of TRAP-positive odontoclasts in the CNT, 14dLi, and 4dLi groups on days 4 and 14. D, dentin; P, pulp; PDL, periodontal ligament; AB, alveolar bone; arrows indicate direction of orthodontic force; scale bars = 100 μm. * $P < .05$, ** $P < .01$.

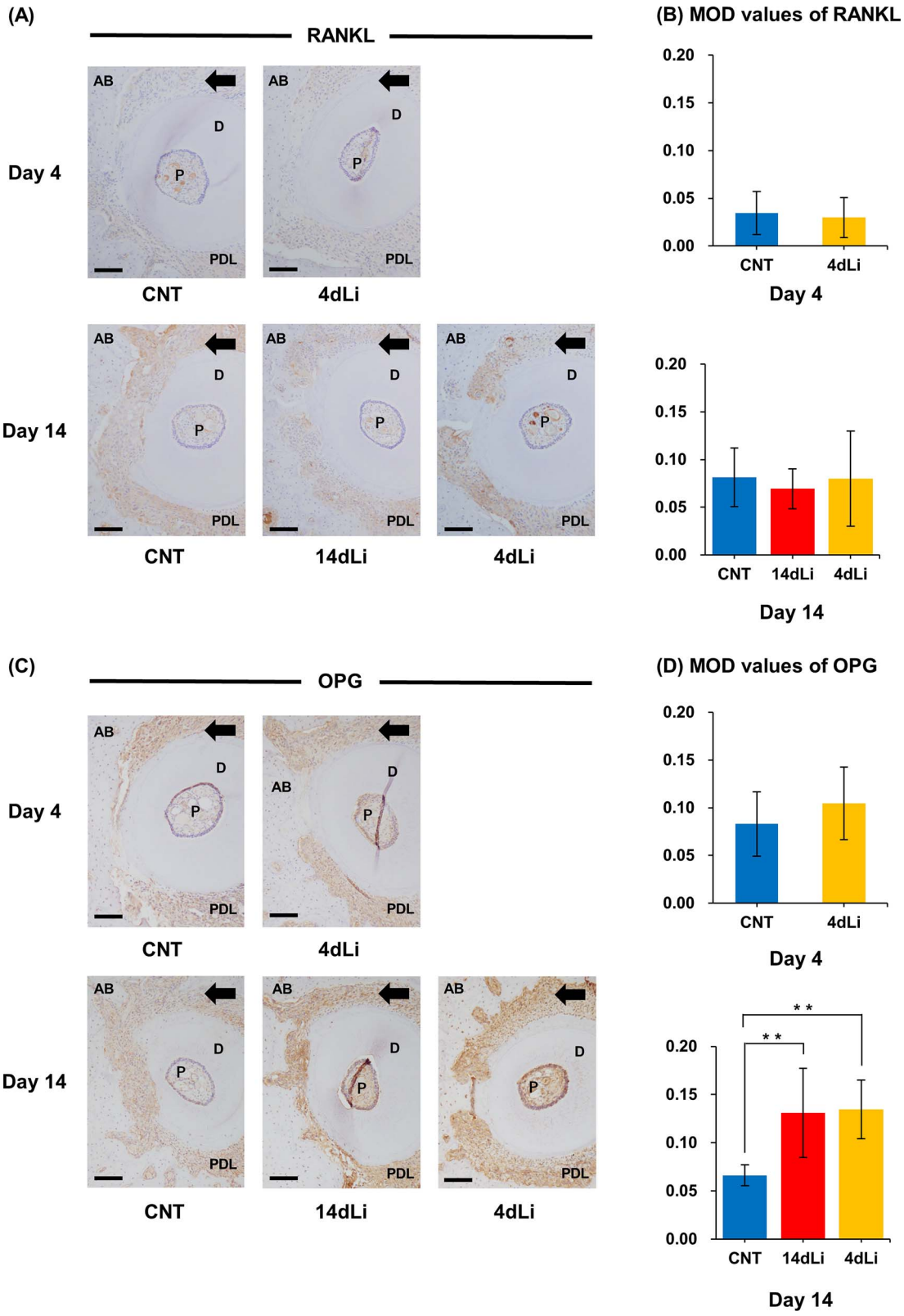

Figure 5. (A) Immunostaining of receptor-activated NF-kB ligand (RANKL) in the control (CNT), 14-day LiCl (14dLi), and 4-day LiCl (4dLi) groups on days 4 and 14 (magnification 20 × 20). (B) The mean optical density (MOD) values of RANKL for the CNT, 14dLi, and 4dLi groups on days 4 and 14. (C) Immunostaining of osteoprotegerin (OPG) in the CNT, 14dLi, and 4dLi groups on days 4 and 14 (magnification 20 × 20). (D) MOD values of OPG for the CNT, 14dLi, and 4dLi groups on days 4 and 14. D, dentin; P, pulp; PDL, periodontal ligament; AB, alveolar bone; arrows indicate direction of orthodontic force; scale bars = 100 μm. ** $P < .01$.

CONCLUSIONS

- Early and short-term Li administration reduced OIRR by increasing OPG expression and suppressing odontoclast differentiation in rats. This highlights the importance of the early phase of OFA in the induction of OIRR.
- Early and short-term Li administration changed the OTM pattern from tipping to bodily movement without reducing the OTM distance in rats.

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