*Original Article*

# Lithium reduces orthodontically induced root resorption by suppressing cell death, hyalinization, and odontoclast formation in rats

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**ABSTRACT**

**Objectives:** To examine whether lithium suppresses orthodontically induced root resorption (OIRR) via two mechanisms (prevention of hyalinization in periodontal tissue and suppression of odontoclasts) and to investigate the changes in the periodontal tissue and alveolar bone, focusing on the appearance of cell death, hyalinization, and odontoclasts.

**Materials and Methods:** The maxillary first molars of 10-week-old male Wistar rats were moved mesially by a closed-coil spring for 14 days. Lithium chloride (LiCl; 0.64 mM/kg) or saline (control) was administered intraperitoneally daily. Tooth movements were measured using micro–computed tomography. Appearances of cell death, hyalinization, and odontoclasts were evaluated by histological analysis.

**Results:** OIRR observed on day 14 in the control group was suppressed strongly by LiCl administration. Apoptotic cells observed on day 1 in the compression area were gradually diminished on days 2 and 3 and transformed to hyalinization tissue in the control group. LiCl administration remarkably suppressed this cell death and subsequent hyalinization. Also, the appearance of odontoclasts in the compression area observed on day 7 was significantly suppressed by LiCl administration. Accordingly, these degenerative processes to OIRR were suppressed substantially by LiCl treatment.

**Conclusions:** Lithium reduces OIRR through the suppression of periodontal ligament cell death, hyalinization, and odontoclast formation. (*Angle Orthod.* 2022;92:547–554.)

**KEY WORDS:** Lithium; Root resorption; Apoptosis; Hyalinization; Odontoclast; Orthodontic tooth movement

## INTRODUCTION

Orthodontic tooth movement (OTM) has great benefits for patients; however, orthodontically induced root resorption (OIRR) has been reported to occur in 90% of teeth moved by this treatment.[1] OIRR is one of the most common and serious adverse effects of OTM and occasionally leads to unsuccessful orthodontic treatment.

Since the first report of Sandstedt,[2] extensive clinical and experimental studies have been conducted on the

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process and mechanism of OIRR and its prevention. It is widely reported that odontoclasts, macrophages, and osteoclasts are involved in OIRR. Studies on these cells have expanded not only to extracellular factors affecting their kinetics such as cytokines and chemokines but also to intracellular signal transduction molecules.[3] Also, the site of OIRR has been reported to correspond to the area of hyalinization of periodontal ligament (PDL) cells compressed by excessive orthodontic force.[4,5] However, the process and mechanism of OIRR has not been elucidated in detail. Accordingly, the prediction of OIRR remains uncertain and preventive methods have not yet been established.[6]

Recently, the administration of lithium was reported to suppress OIRR in rats.[7,8] Lithium has been used for many years to treat bipolar manic depression. However, the underlying mechanisms remain unknown. The most well-known molecular biological function of lithium is the inhibition of glycogen synthase kinase-3β (GSK-3β), which is widely present in cells and regulates various pathways of intracellular signal transduction.[9,10] It is involved in cell proliferation, differentiation, and ischemia-induced cell death.[11] It has been shown that brain cell death attributed to ischemia is suppressed by GSK-3β inhibitors such as lithium.[12] In addition, it was found that activation of GSK-3β in the hypoxic region induces cell death, which is suppressed by lithium.[13,14] Another well-known function is the involvement of lithium in osteoblast differentiation.[15] Patients who took lithium for long periods have been reported to show increased bone mass.[16] It has also been reported that lithium suppresses osteoclast differentiation by suppressing the receptor activator of nuclear factor-κB ligand (RANKL)/osteoprotegerin (OPG) signaling pathway in vivo.[17]

These findings provide a rationale for the exploration of lithium as a potential treatment for OIRR and led to the hypothesis that lithium may suppress OIRR by the following two mechanisms: (1) the prevention of ischemic cell death and hyalinization in PDL tissue and (2) the suppression of odontoclast formation. In this study, the effect of lithium on OIRR through these two mechanisms was examined using an OTM rat model.

## MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of Nagasaki University (No. 2010011668-2).

### OIRR Rat Model

A total of 54 male Wistar rats aged 10 weeks (Japan SLC, Shizuoka, Japan; body weight, 196–218 g) were used in this study. The rats were housed in plastic cages in a colony room and fed a standard pellet diet and water ad libitum. The rats were acclimatized for a week before the experiments. A 25-cN nickel-titanium closed-coil spring (Sentalloy, Tommy, Fukushima, Japan) was placed between the maxillary first molar and the incisors to perform mesial movement of the maxillary first molar according to a previously described method[18] (Figure 1A). This method of fixing the coil spring through the incisors and alveolar bone suppresses tooth eruption and prevents breakage of the appliance. There were a few incidents of appliance breakage and only slight incisor eruption during the experimental period of 14 days. A light-cured composite resin was built on the occlusal surfaces of the second and third molars to eliminate occlusal interferences. All operations were performed under general anesthesia with an intraperitoneal injection of 0.75-mg/kg medetomidine (Domitor, Zenoaq, Fukushima, Japan), 2-mg/kg midazolam (Sandoz, Tokyo, Japan), and 2.5-mg/kg butorphanol tartrate (Meiji Seika Pharma Co Ltd., Tokyo, Japan).

After the appliance was placed, the rats were randomly divided into the following two groups: lithium chloride (LiCl; Wako, Osaka, Japan) group (n = 25) and control group (n = 25). LiCl was dissolved in saline and administered intraperitoneally at concentrations of 0.64 mM/kg of body weight every day in the LiCl group. The same volume of saline was administered to the control group (Figure 1B).

### Micro–Computed Tomography Images and Measurement of OTM

Micro–computed tomography (micro-CT; System R_mCT, Rigaku, Tokyo, Japan) images of live animals under anesthesia were acquired on the day of device attachment (day 0) and days 3, 7, and 14 (Figure 1B). The image acquisition conditions were the following: voltage, 90 kV; current, 100 μA; exposure time, 2 minutes; and resolution, 20 μm/pixel. The following three parameters were defined to measure OTM: (1) shortest distance (ShD), that is, the distance between the distal surface of the maxillary left first molar and the mesial surface of the maxillary left second molar (Figure 1C); (2) distance between contact points (CPD), that is, the distance between the contact points of the maxillary left first and second molars (Figure 1D); and (3) angle of tooth inclination (TIA), that is, the angle between the mesial root axis of the maxillary left first molar and the occlusal plane (Figure 1E).

### Histological Analysis

Rats in the lithium and control groups were euthanized on days 1, 2, 3, 7, and 14 (n = 5 each). The maxilla was dissected and immersed in a fixative

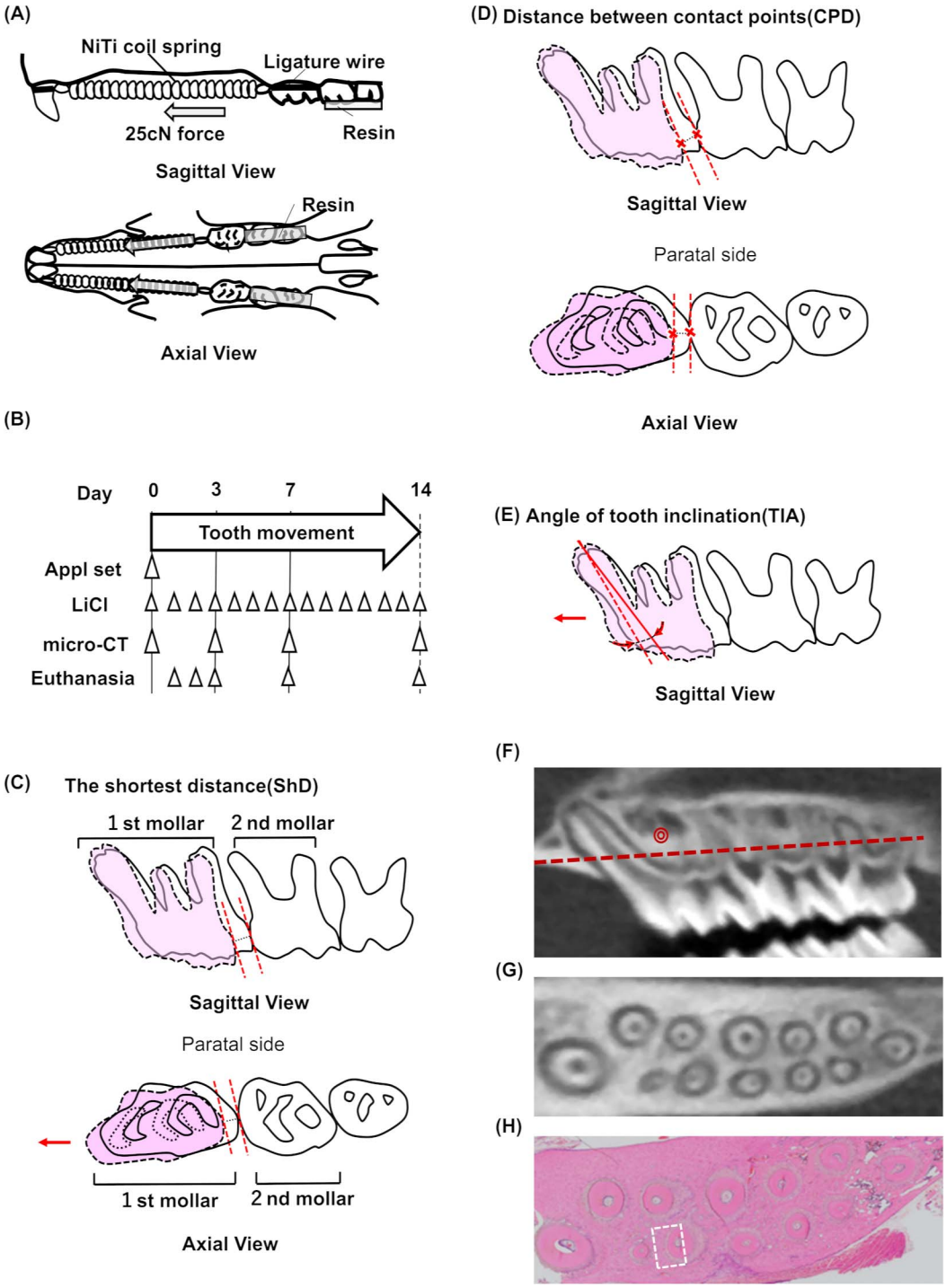


**Figure 1.** Orthodontic appliance, experimental design, measurements of tooth movement, and position of histological analysis. (**A**) Sagittal and axial views of the appliance; arrows indicate the direction of orthodontic force. Gray boxes indicate the light-cured composite resins. (**B**) Experimental time schedule. (**C**–**E**) A method to measure tooth movement: (**C**) ShD, (**D**) CPD, and (**E**) TIA. (**F**) Sagittal micro-CT image of the upper right molars (M1, M2, and M3). The dotted red line indicates the position of the sliced tissue section, which is located at the cervical one-third of the distal root of M1. A double circle indicates the center of rotation. (**G**) An axial micro-CT image of the upper right molars sliced at the level of the dotted line in Figure 1F. (**H**) HE staining of a rat corresponding to the image shown in Figure 1G. The dashed white box indicates the measured area of the distobuccal root of M1. Appl indicates orthodontic appliance.

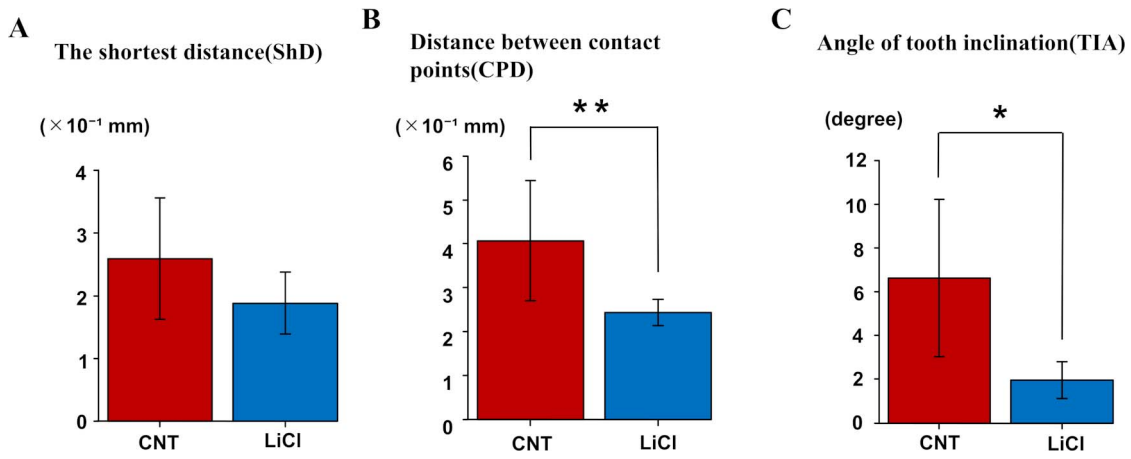


**Figure 2.** Measurements of tooth movement. *$P < .05$ and **$P < .01$ compared with the control group.

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 (EDTA, pH 7.4; Osteosoft, Merck Millipore, Darmstadt, Germany) at 20°C for 4 weeks, dehydrated, and embedded in paraffin. Continuous slicing (6-μm thickness) was performed to observe the cross-sectional structures, and hematoxylin and eosin (HE) staining was performed (Figure 1F–H). The amount of hyalinization and root resorption were measured using ImageJ (National Institutes of Health, Bethesda, Md).

Tartrate-resistant acid phosphatase (TRAP) staining was performed to identify osteoclasts and odontoclasts. Naftol AS-MX phosphate (Sigma, St Louis, Mo), N, N-dimethylformamide (Wako), 0.2-M acetate buffer, and sodium tartrate dihydrate with distilled water stirred and filtered were used to stain at room temperature for 1 hour. The nuclei were stained with hematoxylin.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was performed using the Apoptag Peroxidase In Situ Apoptosis Detection Kit S7100 (Millipore Corporation, Billerica, Mass) according to the manufacturer's protocol.

The numbers of TRAP-positive and TUNEL-positive cells were counted in the mesial periodontium at the distobuccal root of the first molar, one-third from the cervical region of each specimen at a magnification of 20 × 20.

### Statistical Analysis

Statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan). A paired *t*-test was used to test the differences between the two groups. All data are shown as mean ± standard error. A *P* value of less than .05 was considered statistically significant.

## RESULTS

Images from micro-CT scans were used to evaluate tooth movement after the orthodontic force was applied for 14 days (Figure 2). ShD and CPD were slightly smaller in the LiCl group, whereas TIA was significantly suppressed in the LiCl group. These results indicated that tooth movement was observed in both groups; however, the pattern of tooth movement changed from tipping to bodily movement by LiCl treatment.

Hyalinization appeared on the compression side on day 3 (Figure 3A,B). The area of hyalinization was smaller in the LiCl group than in the control group ($0.0045 \pm 0.0019$ mm$^2$ vs $0.0109 \pm 0.0038$ mm$^2$; $P < .05$; Figure 3C). Root resorption was observed on day 14 (Figure 3D,E). The area of root resorption was smaller in the LiCl group than in the control group ($0.0038 \pm 0.0011$ mm$^2$ vs $0.0116 \pm 0.0041$ mm$^2$; $P < .05$; Figure 3F). These findings revealed that the amounts of hyalinization and root resorption were decreased by the administration of LiCl.

TRAP-positive cells on the alveolar bone surface of the compression side were regarded as osteoclasts, and TRAP-positive dentin-faced cells were regarded as odontoclasts. Representative photos on days 3, 7, and 14 are shown in Figure 4A through C and 4E through G. Osteoclasts were found as early as day 3 and declined until day 14, whereas administration of LiCl significantly reduced the appearance of osteoclasts throughout the experimental period ($P < .01$, on days 3 and 7; Figure 4D). In control rats, odontoclasts first appeared on day 7 and increased approximately 1.5 times on day 14, whereas the appearance of odontoclasts was significantly suppressed in LiCl rats ($P < .05$, on day 14; Figure 4H). These results showed that the appearance of osteoclasts preceded the appearance of odontoclasts, and LiCl suppressed the appearance of both cells.

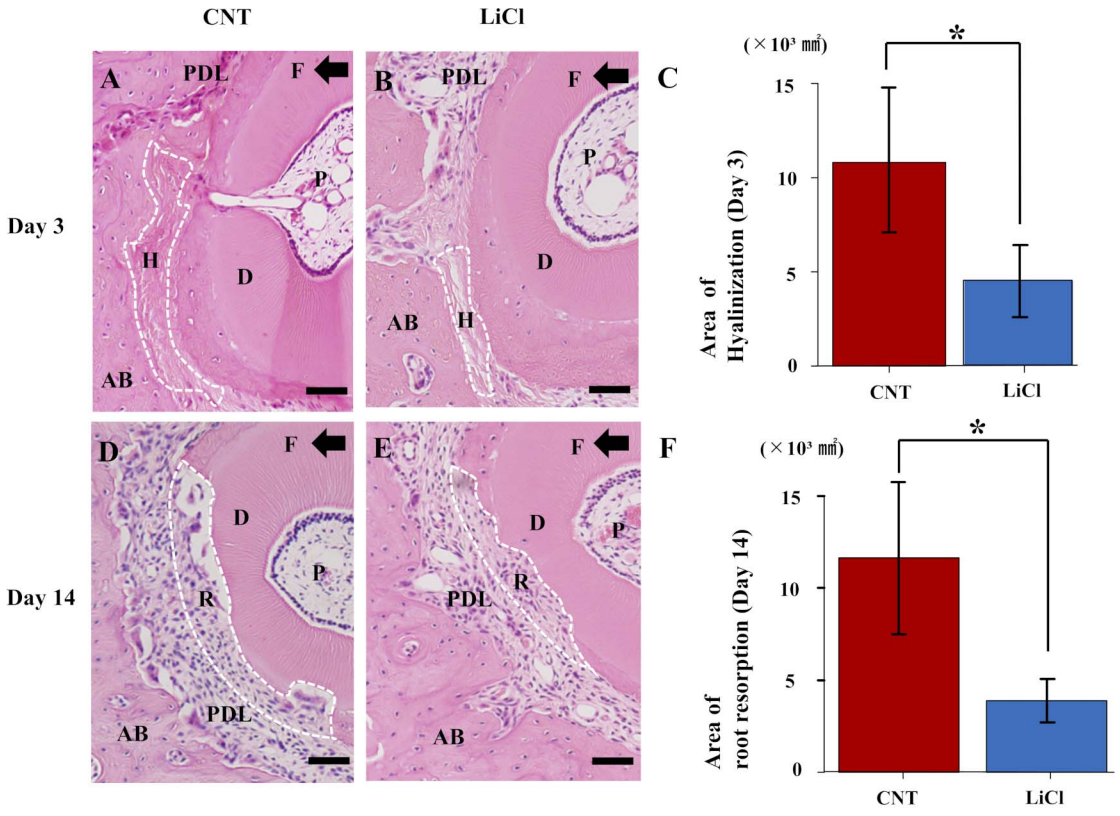


Figure 3

**Figure 3.** HE staining (magnification 20 × 20). Hyalinization of (**A**) control and (**B**) LiCl-administered rats on day 3. The hyalinization area is surrounded by a dotted line. (**C**) The measured area of hyalinization in the PDL. Root resorption in (**D**) control and (**E**) LiCl-administered rats on day 14. (**F**) Measurement values of the area of root resorption craters. Dotted line indicates root resorption lacuna. *$P < .05$ compared with the control group. Scale bars = 50 μm. AB indicates alveolar bone; D, dentine; F, direction of orthodontic force; P, pulp; and R, root resorption lacuna.

TUNEL staining was performed to detect dead or dying cells (apoptotic cells; Figure 5). Apoptotic cells appeared on day 1 in the compression area and gradually decreased on days 2 and 3. Then, hyalinization developed at the same location on day 3 in the control group (Figures 3 and 5). In contrast, the numbers of apoptotic cells were significantly reduced in the LiCl group on day 1 through day 3 (Figure 5G), along with the diminishment of the hyalinization area (Figure 3). These results indicated that TUNEL-positive cells preceded the appearance of hyalinization at the same location in the compressed periodontal tissue, and this series of tissue changes was suppressed by LiCl.

## DISCUSSION

In this study, the histological aspects of the process to OIRR progression were demonstrated. On the compression side of the PDL tissue exposed to ischemia, TUNEL-positive cells (apoptotic cells) were found on day 1, and hyalinization appeared on day 3 at the same location along with the diminishment of apoptotic cells in the control group (Figures 3 and 5). These findings implied that apoptotic cells in the PDL compressed by orthodontic force were subsequently transformed to hyalinization. This is the first report to display the appearance of apoptotic cells and their transformation to hyalinization on the compression side of PDL tissue. Meanwhile, osteoclasts and odontoclasts appeared on days 3 and 7, respectively, and root resorption was observed on day 14 (Figures 3 and 4). These findings suggested that the series of tissue changes provoking OIRR were initiated by ischemia-induced PDL apoptotic cell death and subsequent hyalinization caused by compression of the PDL by orthodontic forces. Then, osteoclasts and odontoclasts appeared, changed the alveolar bone metabolism, and finally proceeded to OIRR.

LiCl showed a strong inhibitory effect on OIRR, as previously reported.[7,8] In addition, in this study, the administration of LiCl suppressed the following two aspects in the process of OIRR: (1) the appearance of apoptotic cells and subsequent hyalinization and (2) the appearance of odontoclasts. Thus, LiCl may

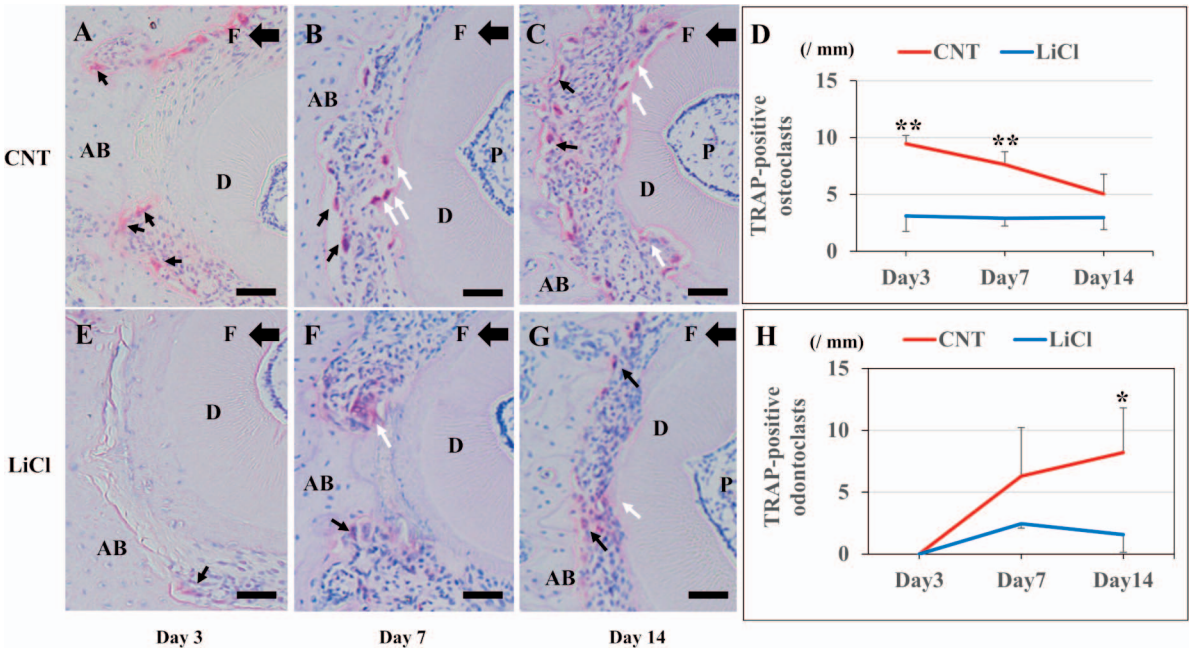


**Figure 4.** TRAP staining of the compression side by the orthodontic force of distobuccal root (magnification 20 × 20) of control (**A**, **B**, **C**) and LiCl (**E**, **F**, **G**). Days 3 (**A**, **E**), 7 (**B**, **F**), and 14 (**C**, **G**) are shown. (**D**) The number of TRAP-positive osteoclasts in the control and LiCl-administered rats. (**H**) The number of TRAP-positive odontoclasts in the control and LiCl-administered rats. White arrows indicate TRAP-positive multinucleate odontoclasts on the dentin and cementum, and black arrows indicate osteoclasts on the alveolar bone. $^{*}P < .05$ and $^{**}P < .01$ compared with the control group. Scale bars = 50 μm.

restrain the progression of OIRR through two mechanisms: first by the suppression of ischemic PDL cell death and subsequent hyalinization, which is an important early pathological condition in OIRR, and second by the suppression of odontoclast and osteoclast kinetics.

Lithium has been suggested to have various intracellular mechanisms. The most well-known molecular biological function of lithium is the inhibition of GSK-3β, which is widely present in cells and regulates various pathways of intracellular signal transduction.[9,10] It has been reported that activation of GSK-3β-induced apoptosis and its inhibition suppressed cell death.[12–14] In this study, the appearance of TUNEL-positive cells in the PDL compressed by orthodontic force was remarkably suppressed by LiCl administration, and the subsequent hyalinization was also remarkably reduced. Because LiCl inhibits apoptosis through the regulation of GSK-3β, this antiapoptotic action of LiCl may have suppressed PDL cell death and reduced hyalinization accordingly.

In addition to its role in cell death, GSK-3β is an important component of Wnt signaling, and its inhibition plays a crucial role in cell proliferation during embryogenesis.[19,20] It is known that GSK-3β inhibition promotes osteoblast differentiation through the Wnt-β-catenin pathway.[21] Lithium enhances bone formation by inhibiting GSK-3β, an enzyme that phosphorylates β-catenin in the cytoplasm. However, calcification by osteoblasts or cementoblasts was not observed in this study. Meanwhile, it was recently reported that lithium suppressed osteoclast differentiation by suppressing the RANKL/OPG signaling pathway in vivo.[17] In this study, LiCl reduced OIRR and suppressed odontoclast formation, which may have been induced by the suppression of apoptosis and hyalinization of PDL cells. Alternatively, it was possible that LiCl acted directly on signal transduction in osteoclasts and odontoclasts to suppress their differentiation.

After 2 weeks of orthodontic force application, the measurement values of ShD and CPD were slightly smaller in the LiCl group than in the control group, whereas TIA was significantly suppressed in the LiCl group (Figure 2). These results indicated that the pattern of tooth movement changed from tipping to bodily movement and suggested that LiCl may have caused differences in bone remodeling between the cervical alveolar bone and apical alveolar bone. This change in tooth movement pattern can be another benefit of lithium on OTM in addition to the suppression of OIRR.

It has been suggested that lithium may affect bone metabolism, an important factor for OIRR. However, two carefully conducted studies indicated that the use of lithium had no adverse effects on bone loss and metabolism.[16,22] Also, lithium has been used clinically for many years, and its effects and adverse effects have been widely recognized. To avoid adverse

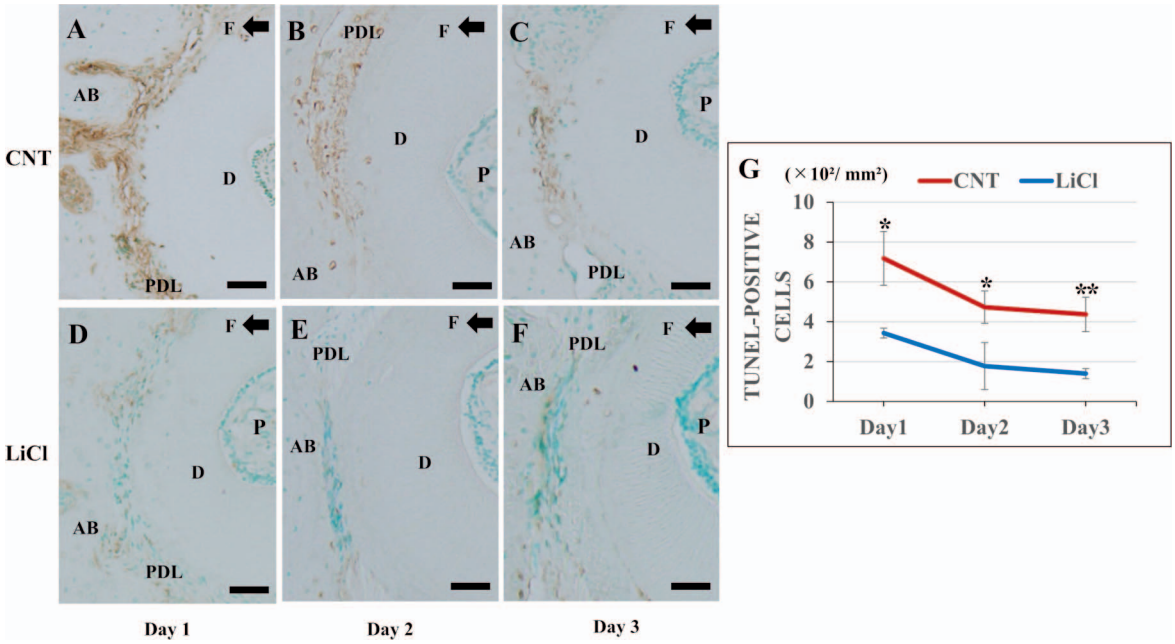


**Figure 5.** TUNEL-positive cells of the control group on days 1 (**A**), 2 (**B**), and 3 (**C**) and of the LiCl group on days 1 (**D**), 2 (**E**), and 3 (**F**) stained brown (magnification 20 × 20). (**G**) The number of TUNEL-positive cells of the control and LiCl groups. *$P < .05$ and **$P < .01$ compared with the control group. Scale bars = 50 μm.

effects, the measurement of blood concentration of lithium has been performed universally. Therefore, the use of lithium could have potential as a preventive measure in orthodontic treatment for patients at risk of OIRR.

## CONCLUSIONS

- LiCl has been shown to reduce OIRR by suppressing cell death (apoptosis) and hyalinization of PDL cells, followed by the reduction of odontoclasts and osteoclasts in rats.
- Thus, further analysis of the action of lithium on OIRR may lead to the prevention and/or the development of preventive medicine for OIRR.

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