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Lithium chloride reduces orthodontically induced root resorption and affects tooth root movement in rats

Airi Ino-Kondo\*; Hitoshi Hotokezaka\*; Takanobu Kondo\*; Keira Arizono\*; Megumi Hashimoto\*; Yuka Hotokezaka\*; Takeshi Kurohama\*; Yukiko Morita\*; Noriaki Yoshida

ABSTRACT

Objective: To investigate the influence of lithium chloride (LiCl) on orthodontic tooth movement (OTM), orthodontically induced root resorption (OIRR), and bone morphometry.

Materials and Methods: Ten-week-old female Sprague Dawley rats (n = 32) were divided into four groups based on the concentration of LiCl administered daily per kilogram body weight: 0 (control group), 0.32, 0.64, and 1.28 mM/kg body weight. The maxillary left first molars were moved mesially by a 10 cN coil spring for 14 days. Micro-computed tomography, scanning electron microscope, and scanning laser microscope images were taken to measure the amount of OTM, the volume of OIRR, and bone morphometry.

Results: OIRR clearly decreased depending on the amount of LiCl administered, although OTM moderately decreased. The tooth inclined mesially and the root apex moved distally in the control and 0.32 mM groups. On the other hand, the tooth inclination angle became smaller and the root apex moved mesially in the 0.64 and 1.28 mM groups. In bone morphometry, the cortical bone mineral content and bone volume increased because of LiCl administration, and the trabecular bone measurements decreased. OIRR negatively correlated to the cortical bone measurements, and the amount of OTM significantly correlated to the cortical bone morphometry.

Conclusions: In rats, LiCl reduced OIRR, which induced mesial movement of the tooth root apex. OIRR positively correlated to cortical bone morphology. (Angle Orthod. 2018;88:474–482.)

KEY WORDS: Root resorption; Tooth root movement; Lithium chloride

INTRODUCTION

Lithium has been commonly prescribed for bipolar manic depression for more than 50 years. The bone density of patients with bipolar disorder who were taking lithium was higher compared with healthy control subjects. Lithium caused an increase in bone volume and bone density by promoting the Wnt/β-catenin signaling pathway in mice. This pathway promotes osteoblastogenesis but inhibits osteoclastogenesis.
Orthodontic tooth movement (OTM) occurs because of a biological response in periodontal tissues caused by an external force. Alveolar bone is resorbed by osteoclasts on the side where the pressure occurs, and new bone is formed by osteoblasts on the side where tension occurs. Various factors have been reported to influence OTM.6,7 Hashimoto et al.8 reported a negative correlation between OTM and bone morphometric measurements, such as trabecular bone structure in ovariectomized rats. It was also reported that tooth movement increased and the optical density of bone decreased on administration of fluoxetine to the rats.9 Therefore, OTM may be greatly influenced by bone morphometric parameters.

Orthodontically induced root resorption (OIRR) is an unavoidable side effect of OTM. Some histological studies have reported that OIRR occurred in 90% of teeth moved by orthodontic treatment.10 Studies have confirmed that OIRR was a part of the hyaline degeneration elimination process by macrophages, osteoclasts, and odontoclasts.11 Hyaline degeneration was reported to be due to cellular apoptosis in rat mesenchymal stem cells.12

To date, no method to control OIRR has been established. Because lithium inhibits the differentiation of osteoclasts,5,13 lithium may affect OIRR and osteoclast differentiation. Interestingly, Wang et al.14 reported that lithium chloride (LiCl) attenuated OIRR in rats. However, the OTM and bone morphometry were not studied in detail. The objective of this study was to evaluate the effects of LiCl on OTM, OIRR, and bone morphology and to determine their correlations.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of Nagasaki University Graduate School of Biomedical Sciences (No. 1012090890). Thirty-two 10-week-old female Sprague Dawley rats (SLC, Shizuoka, Japan; body weight, 194–234 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 acclimatized for a week before the experiments began.

Appliance placement and micro-computed tomography (micro-CT) scanning were performed under general anesthesia induced by an intramuscular injection of 87 mg/kg ketamine hydrochloride (Ketalar 50, Sankyo, Tokyo, Japan) in combination with 13 mg/kg xylazine hydrochloride (Celactal 2%, Bayer-Japan, Tokyo, Japan). A 10 cN nickel-titanium closed-coil spring (Sentalloy, Tomy, Fukushima, Japan) was placed between the incisors and the maxillary left first molar to move the molar mesially. Orthodontic force was applied for 14 days. The occlusal surfaces of the molars, except for the maxillary left first molar, were raised using self-curing resin (Super-Bond, Sun Medical, Shiga, Japan) to eliminate any occlusal force on the maxillary first molar that was moved by orthodontic force (Figure 1A,B).
After the appliance was set, the rats were randomly divided into four groups of eight rats each according to the administered concentration of LiCl (Wako, Osaka, Japan). LiCl was dissolved in saline and administered intraperitoneally to rats daily at concentrations of 0 (control group), 0.32 (0.32 group), 0.64 (0.64 group), or 1.28 mM/kg (1.28 group) of body weight (Figure 1C).

Micro-CT (R_mCT, Rigaku, Tokyo, Japan) images of live animals under anesthesia were acquired on days 0 and 14. The image acquisition conditions were as follows: radiograph source voltage, 90 kV; current, 100 μA; scanning time, 2 minutes; resolution, 20 μm/pixel. Three-dimensional (3D) micro-CT images were superimposed using 3D medical image analysis software (TRI-BONE, Ratoc System Engineering, Tokyo, Japan) to measure the OTM. Four parameters were defined to measure OTM: (1) the shortest distance (ShD), the shortest distance between the distal surface of the maxillary left first molar and the mesial surface of the maxillary left second molar (Figure 2A); (2) distance between contact points (CPD), the distance between the contact points of the maxillary left first and second molars identified on the image on day 0 (Figure 2B); (3) angle of tooth inclination (TIA), the change in tooth inclination defined by the mesial root of the maxillary left first molar and the occlusal plane (Figure 2C); and (4) the distance of movement of the root apex (RAD), the change of distance between the root apex of the mesial root of the first molar and that of the second molar (Figure 2D).

The volume of OIRR was measured as described previously. In short, five roots of the first molars were divided, and the mesial surfaces of the mesial, distobuccal, and distopalatal roots were evaluated in this study. Two-thirds of the cervical side of each root was measured for OIRR. The OIRR craters in the apical third region were not evaluated because the root apices covered by the cellular cementum in rats continuously changed in shape and dimension because of secondary cementum. Scanning electron microscopy (SEM; TM-1000, Hitachi, Tokyo, Japan) was used to separate the border line of OIRR accurately, and a 3D-laser scanning microscope (VK-8500, Keyence, Kyoto, Japan) was used to measure the depth of OIRR. The volume of OIRR was calculated by multiplying the resorption area by the depth.

For bone morphometry, micro-CT images of the right maxilla and left tibia were acquired on days 0 and 14. Bone analysis was subsequently performed using 3D medical image analysis software (TRI-BONE, Ratoc...
System Engineering). A volume of interest (VOI) created from the right maxillary CT image was a cuboidal area located 1.5 mm mesially to the center of the mesial root axis, 3.2 mm apically from the cementoenamel junction including the root apex, and 1.5 mm buccopalatally (Figure 3A). A VOI was created from the proximal tibial CT image located 1.0 mm from the distal end of the growth plate that extended distally up to 2.0 mm (Figure 3B). The following parameters were calculated for each VOI: bone mineral content (BMC), bone volume (BV), bone mineral density (BMD), and tissue volume (TV).

The same investigator performed all measurements, which were repeated three times each. Mean values were used as the final measurements.

**Statistical Analysis**

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan). The data presented a normal distribution (Kolmogorov-Smirnov test, \( P > .05 \) for all data) and homoscedasticity (F-test and Bartlett test, \( P > .05 \) for all data). A one-way analysis of variance was used for each comparison. Tukey tests were used as post hoc tests for intergroup comparisons. Spearman’s rank correlation coefficient was used to evaluate the relationship between LiCl concentration and bone analysis parameters. Pearson’s product–moment correlation coefficient was used to assess the correlation between the bone morphometry parameters and ShD, CPD, RAD, TIA, or OIRR and between the OIRR and ShD, CPD, RAD, or TIA. All data are shown as mean ± standard deviation (SD). \( P < .05 \) was considered statistically significant.

**RESULTS**

There was no significant difference in body weight among all groups on day 0 (217 ± 8 g) and day 14.

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**Figure 4.** Amount of tooth movement (mean ± SD) with control, 0.32, 0.64, and 1.28 mM lithium chloride (LiCl) treatments. (A) Shortest distance (ShD) between the upper first and second molars. (B) Distance between the contact points (CPD). (C) Angle of tooth inclination (TIA). (D) Amount of root apex movement (RAD). * \( P < .05 \); ** \( P < .01 \).
LiCl therapy did not affect weight gain, activity levels, or grooming behavior in the rats. Although there was no significant difference in ShD with administration of LiCl, CPD mildly decreased with LiCl administration. Those in the 0.64 and 1.28 groups were significantly smaller than those in the control group (Figure 4A,B). TIA also decreased depending on the LiCl concentration (Figure 4C). The root apex moved distally by general tipping tooth movement in the control and 0.32 groups; however, the root apex moved mesially in the 0.64 and 1.28 groups (Figure 4D).

In SEM images of the control group, the right first molar roots were usually covered by undamaged cementum with a characteristic smooth surface. In contrast, many OIRR craters were observed on the mesial surface of the roots of the left first molar. In the LiCl group, the OIRR craters were obviously smaller than those of the control group (Figure 5A). The area and depth of OIRR in the LiCl groups tended to decrease compared with those in the control group, and those in the 1.28 group were significantly smaller than those in the control group (Figures 5B,C). The volume of OIRR steeply decreased in a LiCl concentration-dependent manner. The volume of OIRR in the

Figure 5. (A) Typical images of OIRR visualized using SEM. Roots of the control and 1.28 groups on day 14 are shown. Areas of OIRR are surrounded by a broken white line. (B) OIRR area (mean ± SD). (C) OIRR depth (mean ± SD). (D) OIRR volume (mean ± SD). * P < .05.
Figure 6. Ratio of the volume of OIRR and CPD (mean ± SD). * $P < .05$.

Figure 7. Left proximal tibia micro-CT images on day 14. The axial view is the 2-mm slice from the growth plate. The sagittal view is the slice through the middle of the axial view.
To investigate the differential effects of LiCl on OIRR and CPD, the ratios of OIRR volume to CPD among the groups were calculated (Figure 6). The ratios of the 0.64 and 1.28 groups were significantly lower than that of the control group.

To investigate the relationship between the bone and CPD or OIRR, micro-CT images from the upper right alveolar bone and left tibia were used to obtain bone morphometry. Because the alveolar bone was too small to obtain trabecular bone morphology, the left tibia was used. According to the micro-CT images of the left tibia, cortical bone thickness was greater in the 0.64 group than in the control group (Figure 7). Cortical BMC and cortical BV tended to be greater in all LiCl groups compared with the control group in both the alveolar and tibia locations. Trabecular BMC, trabecular BV, and trabecular TV tended to be smaller in the LiCl groups compared with the control group in the results of the tibia (Table 1). Cortical BMC, BV, and BMD in the alveolar bone and cortical BMC and BV in the tibia showed a significant positive correlation with LiCl concentration. These parameters tended to increase depending on the concentration of LiCl. However, trabecular BMC, BV, and TV in the tibia tended to decrease depending on the concentration of LiCl, although the differences were not significant in trabecular BMC and BV (Table 2).

ShD, CPD, TIA, and OIRR negatively correlated with cortical BMC and BV in the alveolar bone and tibia. However, ShD positively correlated with trabecular TV. On the other hand, RAD positively correlated with cortical BMC and BV in the alveolar bone and tibia and negatively to the trabecular TV of the tibia (Table 3). OIRR was significantly positively correlated with ShD, CPD, and TIA and significantly negatively correlated with RAD (Table 4).

### DISCUSSION

In this study, LiCl reduced OIRR with mild reduction of OTM in rats, and increased cortical BMC and BV and trabecular TV. This result might have occurred because LiCl promoted not only osteoblastogenesis but also osteoclastogenesis and odontoclastogenesis by promoting the Wnt/β-catenin signaling pathway.

The experimental model that applied a mesial force to the upper first molar in rats in this study is often used to investigate OTM and OIRR, and it generally induces tipping tooth movement. Interestingly, the mesial root apex of the first molar moved mesially in the 0.64 and 1.28 groups, although it moved distally in the control and 0.32 groups, whereas RAD positively correlated to cortical bone morphometry and negatively with trabecular bone morphometry. These results suggested that the augmented cortical bone surrounding the cervical region suppressed ShD, CPD, and TIA and decreased trabecular bone surrounding the root-enhanced RAD.

LiCl strongly reduced the area, depth, and volume of OIRR, and subsequently, the ratio of OIRR per CPD was significantly smaller in the 0.64 and 1.28 groups than in the control group. These results suggested that LiCl inhibited OIRR more efficiently than OTM. Furthermore, OIRR has a negative correlation with RAD and has a positive correlation with TIA, which suggested that LiCl induced mesial root movement that led to increased bodily movement of the tooth, which

### Table 1. Bone Analysis Parameters

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<th>Tibia</th>
<th>Trabecula</th>
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<td>BMC BV BMD</td>
<td>BMC BV BMD</td>
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<td>Control</td>
<td>2.33 ± 0.09 2.62 ± 0.12 889 ± 23</td>
<td>10.5 ± 0.95 14.8 ± 1.6 711 ± 22</td>
<td>1.22 ± 0.32 2.63 ± 0.66 461 ± 6</td>
<td>16.5 ± 2.1</td>
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<tr>
<td>0.32</td>
<td>2.47 ± 0.21 2.76 ± 0.19 892 ± 26</td>
<td>11.3 ± 1.2 16.1 ± 1.8 702 ± 19</td>
<td>1.15 ± 0.21 2.50 ± 0.45 461 ± 5</td>
<td>15.8 ± 1.9</td>
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<tr>
<td>0.64</td>
<td>2.57 ± 0.14 2.84 ± 0.16 906 ± 22</td>
<td>12.6 ± 1.8* 18.0 ± 2.8* 700 ± 25</td>
<td>1.04 ± 0.28 2.25 ± 0.56 460 ± 13</td>
<td>13.1 ± 2.3*</td>
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<tr>
<td>1.28</td>
<td>2.85 ± 0.27** 3.16 ± 0.28** 902 ± 16</td>
<td>11.6 ± 1.2 16.3 ± 2.3 717 ± 30</td>
<td>1.03 ± 0.17 2.25 ± 0.36 456 ± 6</td>
<td>14.5 ± 3.3</td>
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</table>

* BMC indicates bone mineral content (mg); BV, bone volume (×10^3 cm³); BMD, bone mineral density (mg/cm³); TV, trabecular tissue volume (×10^-3 cm³); ** P < .05; *** P < .01 compared with the control group.

### Table 2. Spearman’s Rank Correlation rho of LiCl Concentration and Bone Morphometry

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<th>Tibia</th>
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<td>BMC BV BMD</td>
<td>BMC BV BMD</td>
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<td>Correlation coefficient</td>
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<td>.44 .39 .04</td>
<td>-.31 -.32 -.19</td>
<td>-.37</td>
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<td>P value</td>
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<td>&lt;.01** .02* .01</td>
<td>.07 .06 .29</td>
<td>.03*</td>
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* BMC indicates bone mineral content; BV, bone volume; BMD, bone mineral density; TV, trabecular tissue volume; ** P < .05; *** P < .01.
may have subsequently been partially involved in the reduction of OIRR. Alternatively, these differential effects of LiCl on OTM and OIRR may have been caused by other mechanisms. As OIRR occurs beneath the areas of hyaline degeneration, the area of OIRR is influenced by the range of the degenerated tissue.11 Because lithium also inhibits apoptosis through regulation of GSK-3β in various cellular responses,17 the antiapoptotic action of LiCl may have decreased the range of hyaline degeneration, which reduced the OIRR area in this study. In addition, LiCl reduced the depth of OIRR, which may have been due to the inhibition of odontoclast differentiation and activation by lithium, similar to osteoclasts.18,19 These combined actions of LiCl on periodontal tissue may have greatly decreased the volume of OIRR.

In this study, LiCl decreased OTM and OIRR. Although many previous reports support the results of this study, further investigation is necessary to determine the in vivo mechanism. Lithium has been used as a therapeutic agent for bipolar disorder; nevertheless, the mechanisms of how lithium acts as a multifunctional molecule with a wide range are still unknown.

Therefore, lithium is never currently applied to orthodontic treatment. Further study is necessary to elucidate the mechanism of lithium to suppress OIRR, which may be able to control orthodontic treatment more safely.

CONCLUSIONS

- LiCl reduced OIRR, which induced mesial movement of tooth roots in rats.
- OIRR positively correlated to cortical bone morphometry.

ACKNOWLEDGMENTS

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REFERENCES

4. Bain G, Muller T, Wang X, Pankoff J. Activated beta-catenin induces osteoblast differentiation of C3H10T1/2 cells and...