Original Article

Local administration of high-dose diabetes medicine exendin-4 inhibits orthodontic tooth movement in mice

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ABSTRACT

Objectives: To investigate the effects of exendin-4 on orthodontic tooth movement distance, root resorption, and expression levels of osteoclast-related cytokines in a mouse model.

Materials and Methods: A 10-g NiTi coil spring was placed between the anterior alveolar bone and upper left first molar of 8-week-old male C57BL/6 mice. Twenty microliters of exendin-4 solution (containing 0.2 μg, 4 μg, or 20 μg exendin-4) or phosphate-buffered saline (PBS) were injected on the buccal side of the upper left first molar at 2-day intervals (4 mice per group). Mice were sacrificed on day 12; silicone impressions were taken to record tooth movement distance. The left maxillae of the PBS and 20 μg exendin-4 groups were also excised for histological analysis and quantitative reverse transcription polymerase chain reaction analysis.

Results: Orthodontic tooth movement distance was smaller in the 20 μg exendin-4 group than in the PBS group ($P < .01$). Compared with the PBS group, the 20 μg exendin-4 group showed lower osteoclast number ($P < .05$), odontoclast number ($P < .05$), and root resorption surface percentage ($P < .05$). Relative to maxillae with PBS injections, maxillae with 20 μg exendin-4 injections had lower receptor activator of nuclear factor kappa-B ligand (RANKL) mRNA expression ($P < .05$), TNF-α mRNA expression ($P < .05$), and RANKL/osteoprotegerin (OPG) ratio ($P < .01$). There were no differences in the expression of OPG mRNA.

Conclusions: Exendin-4 inhibits orthodontic tooth movement. Therefore, additional attention is needed for orthodontic patients who receive exendin-4 for diabetes treatment. GLP-1 receptor may be a treatment target for patients with severe root resorption. (*Angle Orthod.* 2021;91:111–118.)

KEY WORDS: Diabetes; GLP-1; Exendin-4; Osteoclast; Orthodontic tooth movement

INTRODUCTION

The prevalence of type 2 diabetes is increasing worldwide, having approached 8.8% of the adult population in 2017.[1] Glucagon-like peptide-1 (GLP-1) receptor agonists, which resemble the intestinal GLP-1 hormone but exhibit greater resistance to the degradation of dipeptidyl peptidase-4 enzyme, have been developed to treat type 2 diabetes.[2] GLP-1 receptor agonists function by preventing apoptosis of pancreatic β cells and stimulating insulin release by those cells.[3–5] Because of the broad expression of GLP-1 receptors in various cells, the effects of these receptors, beyond blood glucose control, have been investigated recently.[6] GLP-1 receptor agonists can also suppress chronic inflammation of multiple organs by downregulating the expression levels of inflammatory cytokines (eg, interleukin-1, interleukin-6, and tumor necrosis factor-α [TNF-α]).[7] Exendin-4, mainly administered by subcutaneous injections, is reportedly the most potent GLP-1 receptor agonist that can reduce the bone fracture rate of diabetic patients.[8]

Orthodontic tooth movement is a dynamic process achieved through bone resorption by osteoclasts on

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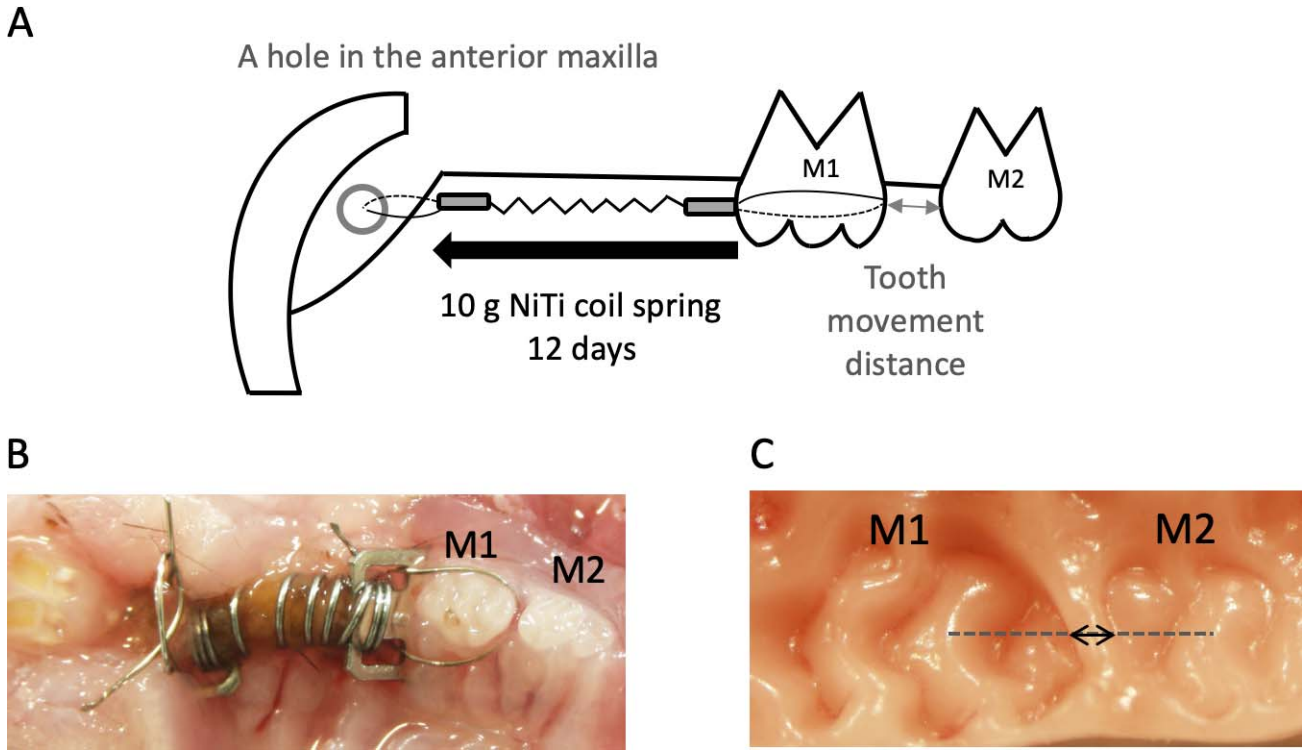

Figure 1. Measurement of orthodontic tooth movement in mice. (A) Appliance design. (B) An intraoral photograph after 12 days of orthodontic tooth movement. A gap between upper left first (M1) and second molars (M2) is visible. (C) The gap (double arrow) between M1 and M2 on the line (dashed line) connecting the central fossae of the two molars was measured using silicone impressions.

the compression side and bone apposition by osteoblasts on the tension side. During this process, root-resorbing odontoclasts appear alongside osteoclasts. Receptor activator of nuclear factor kappa-B ligand (RANKL)[9,10] and TNF-α[11,12] are crucial for differentiation of osteoclasts and odontoclasts during orthodontic tooth movement. Exendin-4 can inhibit TNF-α expression[13,14] and TNF-α-induced inflammation or reactions.[15–17] Increased levels of RANKL expression in the bone of osteoporotic rodents have been ameliorated by administration of exendin-4.[18–20] Therefore, the present study investigated the effects of exendin-4 on orthodontic tooth movement and associated root resorption in a mouse model.

MATERIALS AND METHODS

Animals and Reagents

Eight-week-old male C57BL6/J mice were obtained from CLEA Japan (Tokyo, Japan). For local injections, various quantities of exendin-4 (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in phosphate-buffered saline (PBS) to achieve the necessary doses. All experiments were approved by the animal ethical review committee of Tohoku University. Four mice were included in each group.

Distance of Orthodontic Tooth Movement

Mice were anesthetized, then exposed to orthodontic force exerted by a 10-g closed nickel–titanium (NiTi) spring tied between the upper left first molar and a hole in the anterior alveolar bone by ligature wires (Figure 1A,B). Twenty microliters of exendin-4 solution (0.2, 4, or 20 μg dissolved in 20 μl PBS) or PBS were injected on the buccal side of the upper left first molar on days 0, 2, 4, 6, 8, and 10. To facilitate ingestion of food, mice were fed a powder diet (CLEA Japan). Mice were sacrificed on day 12. Polysiloxane impressions (Examixfine, GC, Tokyo, Japan) were then taken to measure the distance of tooth movement. Distance was measured between the distal marginal ridge of the first molar and mesial marginal ridge of the second molar on the line connecting the central fossae of the first and second molars (Figure 1C) using a dissecting microscope (Leica M165FC, Leica, Wetzlar, Germany).[21]

Histological Quantification of Osteoclasts

Maxillae were fixed in 4% formaldehyde in PBS and decalcified with OSTEOSOFT solution (Sigma-Aldrich). Samples were processed using a tissue processer (Leica TP1020, Germany), then embedded in paraffin. Horizontal sections were cut at 100, 140, 180, 220, and 260 μm apical to the bifurcation area of

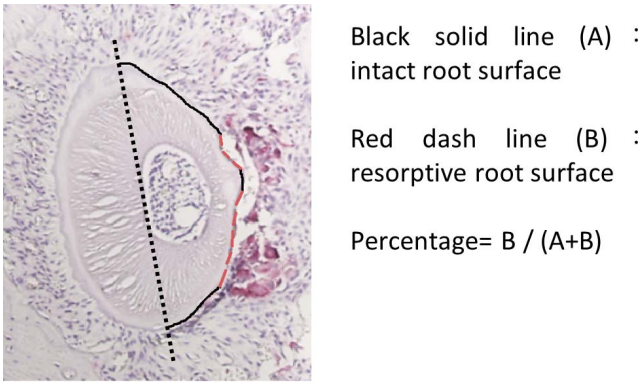

Figure 2. Histological quantification of root resorption surface percentage. Root resorption surface percentage (%) was calculated by dividing the length of the resorption surface by the whole length of the root surface on the compression side. R indicates root; P, PDL.

the upper left first molar. Sections were stained with tartrate-resistant acid phosphatase (TRAP) solution and counterstained with hematoxylin. The labiolingual long axis of the distobuccal root was drawn; the area mesial to this axis was regarded as the compression side. The number of large TRAP-positive osteoclasts was measured on the alveolar surface of the compression side.[21]

Histological Quantification of Odontoclasts and Root Resorption

The number of TRAP-positive odontoclasts was measured on the root surface of the compression side. Thinning or lacunae of cementum with overlying odontoclasts was regarded as resorptive root surface. Lengths of intact root surface and resorptive root surface on the compression side were measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Root resorption surface percentage (%) was the ratio of resorptive root surface to whole root surface (the sum of intact and resorptive root surface) on the compression side (Figure 2).[21]

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) Analysis

The left maxilla around the upper left first molar was immersed in TRIzol reagent, then crushed using beads and Micro Smash MS-100R (Tomy Seiko, Tokyo, Japan) (Invitrogen, Carlsbad, CA, USA). Total RNA was extracted using a RNeasy mini kit (Qiagen, Valencia, CA, USA). Reverse transcription of cDNA from total RNA was performed according to the protocol of the SuperScript IV first-strand cDNA synthesis system (Invitrogen). mRNA expression levels of RANKL, osteoprotegerin (OPG), and TNF-α, relative to glyceraldehyde 3-phosphate dehydrogenase, were measured by qRT-PCR using a Thermal Cycler Dice Real Time system (Takara, Shiga, Japan). The primers were described previously.[14]

Statistical Analysis

All data were expressed as mean $\pm$ standard deviation. Comparisons were performed using Student's *t*-test or analysis of variance, followed by post hoc Scheffe's tests. Differences with $P < .05$ were considered statistically significant. All statistical analyses were conducted with SPSS Statistics software (IBM Corp., Armonk, NY, USA).

RESULTS

On day 12, the mean tooth movement distance in the PBS group was 184.5 $\pm$ 8.7 μm. In the 0.2 μg, 4 μg, and 20 μg exendin-4 groups, the mean distances of tooth movement were 179.25 $\pm$ 11.2 μm, 163.3 $\pm$ 13.1 μm, and 137.8 $\pm$ 6.2 μm, respectively. Tooth movement distance was significantly smaller in the 20 μg exendin-4 group than in the PBS group (25.3% reduction, $P < .01$). However, there were no significant differences among the PBS injection, 0.2 μg exendin-4 injection, and 4 μg exendin-4 groups (Figure 3).

Mean numbers of TRAP-positive osteoclasts in the PBS and 20 μg exendin-4 groups were 12.1 $\pm$ 3.2 and 7.5 $\pm$ 2.3, respectively. The 20 μg exendin-4 group exhibited a lower osteoclast number on the compression side compared with the PBS group (38.1% reduction, $P < .05$) (Figure 4).

The PBS group had severe root resorption, as demonstrated by high odontoclast number (3.0 $\pm$ 1.7) and root resorption surface percentage (19.8% $\pm$ 8.8%). Mice injected with 20 μg exendin-4 exhibited lower odontoclast number (0.9 $\pm$ 0.8, 71.2% reduction, $P < .05$) (Figure 5A) and root resorption surface percentage (5.9% $\pm$ 4.2%, 70% reduction, $P < .05$) (Figure 5B), relative to mice injected with PBS.

Maxillae injected with 20 μg exendin-4 had lower mRNA expression levels of RANKL (66.3% reduction, $P < .05$) (Figure 6A) and TNF-α (45.5% reduction, $P < .05$) (Figure 6B), relative to maxillae injected with PBS during orthodontic tooth movement. Although there were no differences in the expression of OPG mRNA (Figure 6C), the RANKL/OPG ratio (72.9% reduction, $P < .01$) (Figure 6D) was reduced in exendin-4 groups, compared with the PBS group.

DISCUSSION

GLP-1 receptors are expressed in osteoclasts, osteocytes, and osteoblasts.[22] The effects of GLP-1 receptor agonists on bone metabolism have been widely explored recently. Because the rate of ortho-

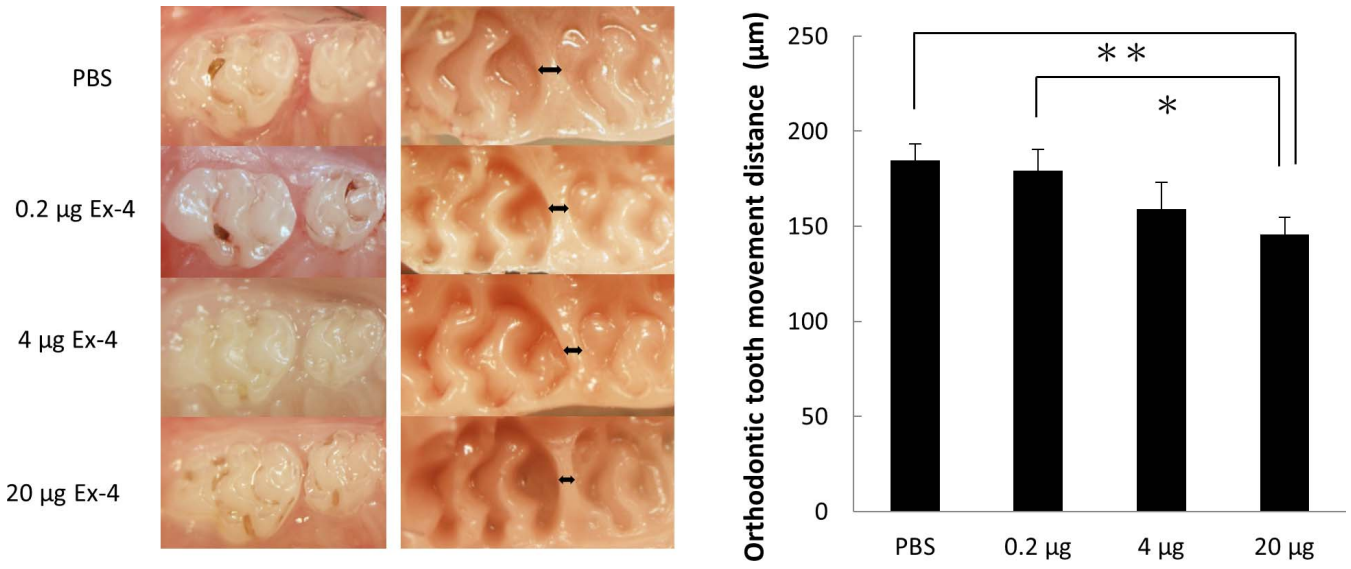

Figure 3. Dose-dependent effects of exendin-4 (Ex-4) injections on orthodontic tooth movement distance on day 12. Silicone impressions and tooth movement distances of mice injected with 20 μl PBS or solutions of 0.2 μg, 4 μg, or 20 μg exendin-4 at 2-day intervals (4 mice per group). *$P < .05$, **$P < .01$.

dontic tooth movement is closely related to the rate of alveolar bone turnover,[23] this study investigated the effect of a GLP-1 receptor agonist on orthodontic tooth movement.

GLP-1 receptor-deficient mice reportedly exhibit osteopenia and elevated osteoclast formation, suggesting that the GLP-1 receptor signaling pathway has an anti-resorptive effect on bone metabolism.[24,25] Exendin-4 improves diabetes-induced, ovariectomy-induced, and high fat diet-induced osteoporosis in rodent models.[18–20,22,26,27] Underlying mechanisms may include reduction of the RANKL/OPG ratio[18–20] and TNF-α expression level[14] in bone tissue, as well as reduction of serum calcitonin level.[22,24] A direct effect of exendin-4, via GLP-1 receptors on osteoclasts, may not be possible because minimal in vitro effects have been observed.[14,22] Subcutaneous injections of exendin-4 increased bone formation markers and in vivo osteoblast number of ovariectomized rats.[20] Additionally, exendin-4 promoted differentiation of bone marrow

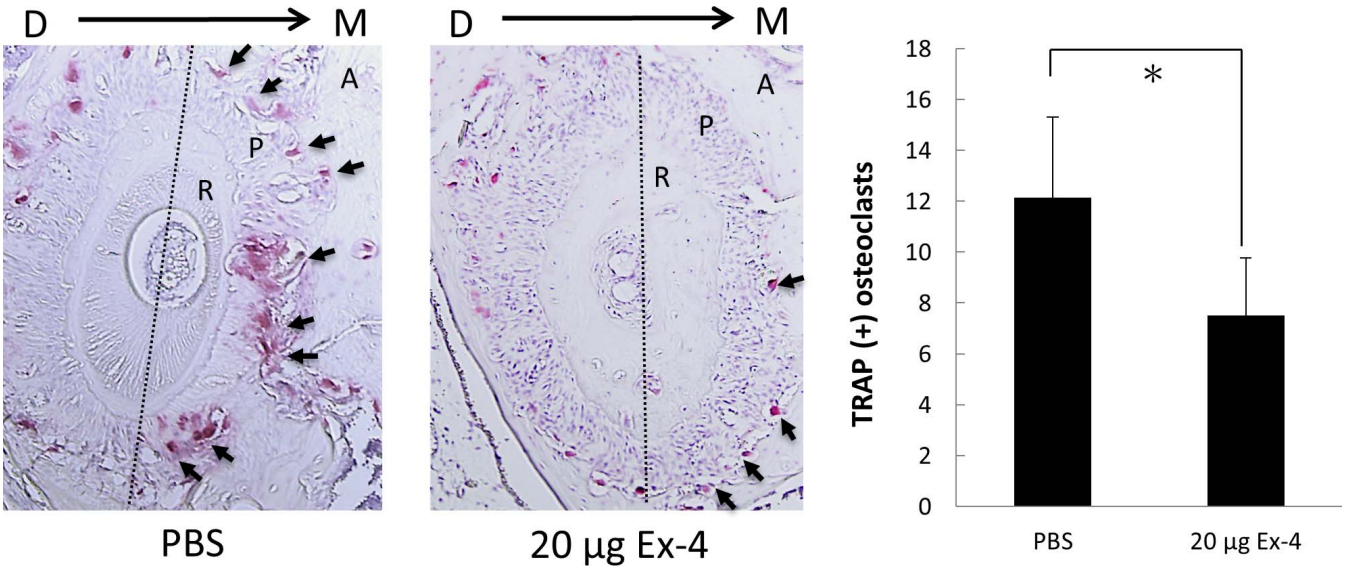

Figure 4. Histological evaluation of osteoclast numbers after 12 days of orthodontic tooth movement. Maxillae injected with PBS or 20 μg exendin-4 were obtained for preparation of histological sections (4 mice per group). Large TRAP (+) osteoclasts (arrow) appeared on the alveolar bone surface mesial to the buccolingual root axis (dashed line) of the distobuccal root of M1. D indicates distal; M, mesial; R, root; P, PDL; A, alveolar bone. *$P < .05$.

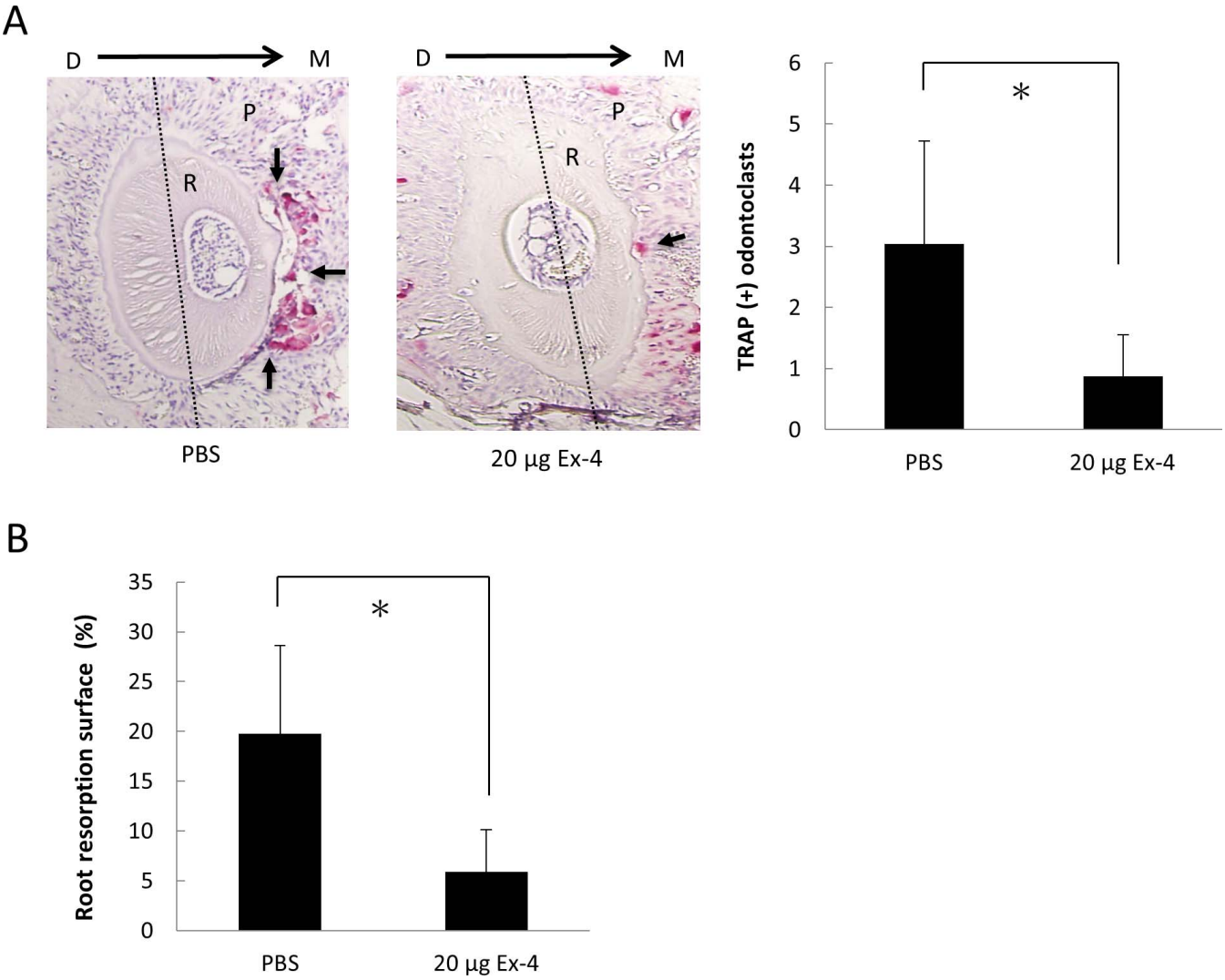

Figure 5. Histological evaluation of odontoclast numbers and root resorption surface percentages after 12 days of orthodontic loading. (A) TRAP (+) odontoclasts (arrow) resting on the distobuccal root surface of M1 in PBS and exendin-4 groups (4 mice per group). (B) Root resorption surface percentage (%). (4 mice per group). D indicates distal; M, mesial; R, root; P, PDL. *$P < .05$.

stromal cells into osteoblasts by synergizing with the Wnt signaling pathway.[28] In alveolar bone, exendin-4 regulates Wnt and NF-κB signaling in the osteogenic differentiation of periodontal stem cells during inflammation.[29] Therefore, exendin-4 can downregulate bone resorption and upregulate bone formation. Medicines and cytokines that inhibit osteoclasts have been shown to reduce orthodontic tooth movement.[11,21,30] In the present study, high doses of the diabetes medicine, exendin-4, inhibited orthodontic tooth movement and osteoclast formation on the compression side. Previous rodent studies showed that long-term subcutaneous injection of low-dose exendin-4 (<0.2 μg/d) improved ovariectomy-induced osteoporosis.[20,22] However, orthodontic tooth movement did not significantly change in the 0.2 μg and 4 μg exendin-4 groups, presumably because of the short-term injections in this study. Therapeutic doses of exendin-4 for diabetic patients, which are as low as the dose in the 0.2 μg group, are unlikely to significantly affect orthodontic tooth movement in the short term. Short-term injections of 20 μg exendin-4 have been shown to ameliorate LPS-induced bone resorption on murine calvariae.[14] Although tissue reactions induced by orthodontic tooth movement were less robust than reactions induced by LPS, inhibition of orthodontic tooth movement required high doses of exendin-4. Small sample sizes in all groups and poor distributions of exendin-4 in alveolar bone after local injections may have contributed to these results.

Root resorption is inevitable during orthodontic treatment.[31] In this study, the PBS group exhibited severe root resorption because of sustained heavy loading force. However, high-dose exendin-4 effective-

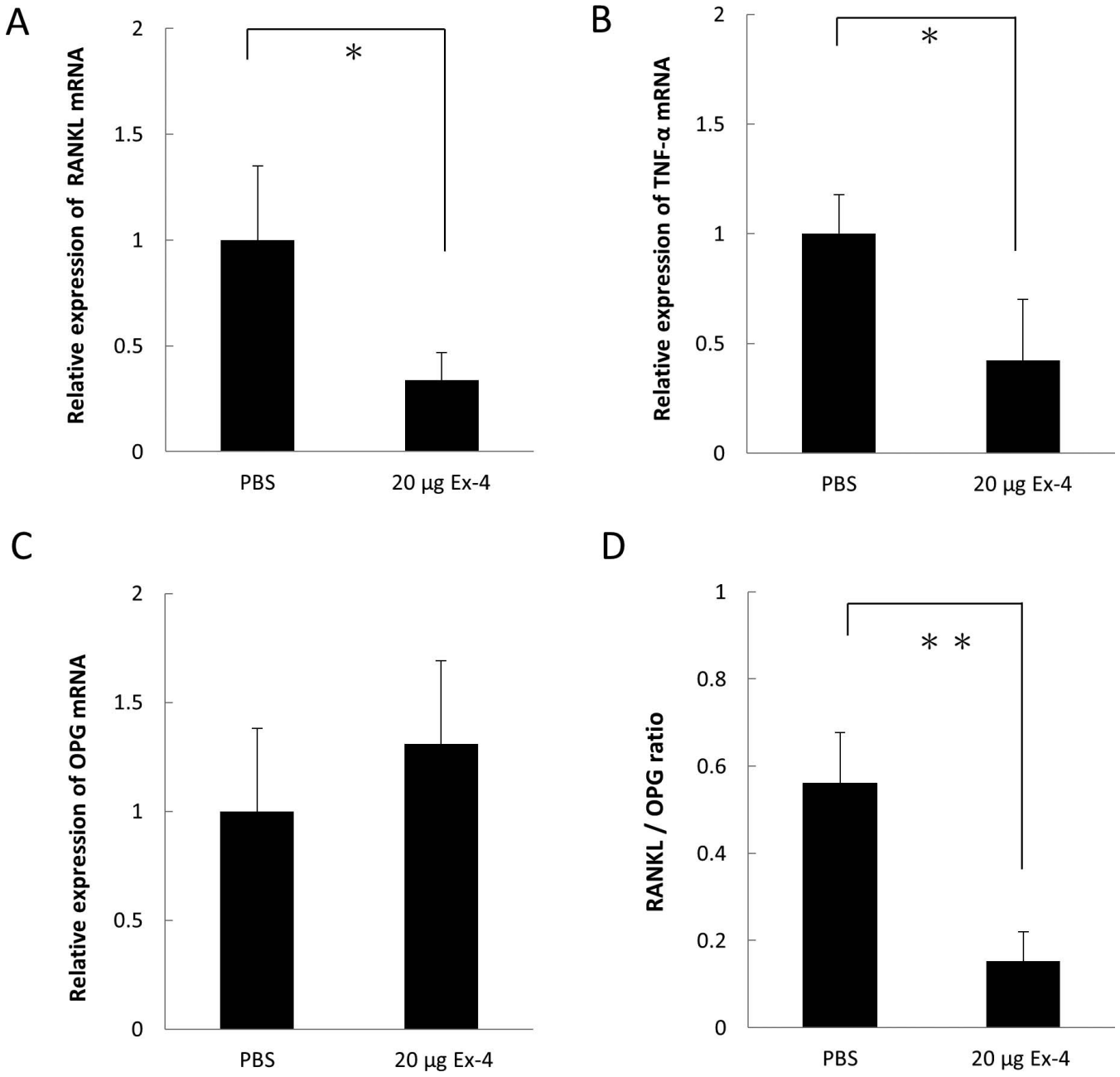

Figure 6. Effect of local exendin-4 injections on RANKL mRNA (A); TNF-α mRNA (B); OPG mRNA (C); and RANKL/OPG ratio (D) of maxillae during orthodontic tooth movement. Maxillae with injections of PBS or 20 μg exendin-4 solutions were excised for qRT-PCR analysis after 12 days of orthodontic loading (4 mice per group). * $P < .05$. ** $P < .01$.

ly prevented this phenomenon, which indicated the potential of the GLP-1 receptor agonist in treating orthodontic root resorption.

Histological analyses revealed reduced osteoclast number, odontoclast number, and root resorption surface percentage in the 20 μg exendin-4 group, compared with the PBS group. Because both RANKL and TNF-α are responsible for the differentiation of osteoclasts and odontoclasts, expression levels of these cytokines in maxillae were measured by qRT-PCR. Consistent with the histological results, RANKL and TNF-α mRNA expression levels in maxillae were reduced by injection with 20 μg exendin-4. Exendin-4 reportedly blocks TNF-α expression by inhibiting intracellular NF-κB signaling.[7] Exendin-4 also directs macrophage polarization toward the M2 phenotype; therefore, expression levels of M1 macrophage cytokines (eg, TNF-α) are reduced.[32] Subcutaneous administration of exendin-4 for 3 months reportedly reduced RANKL expression, increased OPG expres-

sion, and reduced the RANKL/OPG ratio in ovariectomized rats.[20] OPG is an inhibitor of osteoclasts and orthodontic tooth movement.[33] Although OPG mRNA expression increased slightly in exendin-4 groups, it was not significantly affected by exendin-4 in this study. Further studies with longer durations of exendin-4 treatment are needed to ascertain its effects on OPG expression during orthodontic tooth movement.

CONCLUSIONS

- Exendin-4 inhibits osteoclast formation and tooth movement during orthodontic loading. Therefore, additional monitoring is needed for orthodontic patients taking exendin-4 for diabetes treatment.
- Further clinical application of exendin-4 in treating root resorption is possible due to its negative effect on odontoclasts.

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