Jiggling Force Induces Orthodontic Root Resorption during Tooth Movement in Rats

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Abstract
Orthodontic root resorption (ORR) is an unavoidable pathological consequence of orthodontic tooth movement. It is thought that swinging of the root due to the reciprocating movement of the tooth (jiggling) may exacerbate ORR. However, little is known about the relationship between ORR and jiggling. We herein investigated the tartrate-resistant acid phosphatase (TRAP) expression in odontoclasts in resorbed roots during experimental tooth movement (jiggling) in vivo.

Twenty-four eight-week-old male Wistar rats were divided into four groups; a heavy force group (50 g), an optimal force group (10 g), a jiggling force group (compression and tension, repetition; 10 g) and a control group. The expression levels of TRAP protein in odontoclasts in the dental root were determined by immunohistochemical analysis.

Immunoreactivity for TRAP in resorbed roots exposed to the jiggling force was stronger than that in the other groups on day 21. The number of TRAP-positive odontoclasts was significantly elevated in the JF group on day 21 when compared with the other groups.

These results suggest that “jiggling force” may induce ORR during orthodontic tooth movement, and may be a risk factor for ORR.

Keywords:
experimental tooth movement, jiggling force, root resorption, orthodontic force

Introduction
Orthodontic treatment has many advantages, including improvements in masticatory ability (1) and the ability to restore aesthetics; however, there are risks associated with these procedures. For example, orthodontic root resorption (ORR) is one of the most difficult procedure-related adverse events to predict in cases of orthodontic tooth movement; this undesirable result can cause permanent loss of the dental structure of the root apex. In an epidemiological study by Kaley et al. (2), all patients who underwent comprehensive orthodontic treatment presented with root shortening, and 3% of all patients had severe root resorption (shortening by more than one-quarter of the root length) with root shortening in the maxillary central incisors. There are many factors involved in the process of root resorption (3). The causes of this phenomenon have been reported to include the use of heavy force (4), length of treatment (3), type of root (5), and genetic predisposition (6). In particular, numerous studies have discussed the relationship between heavy force and ORR (4, 7).

Furthermore, recent studies reported that heavy force induced ORR via the expression of receptor activator of nuclear factor-kB (RANK)/RANK ligand (RANKL), interleukin (IL)-6, IL-8, and IL-17 from PDL cells (8, 9, 10).

An orthodontist encounters ORR when teeth (particularly maxillary anterior teeth) are moved by a jiggling force during orthodontic treatment. A “jiggling” force is that which moves the roots of teeth mesiodistally or buccolingually during orthodontic treatment. It is thought that a jiggling movement may induce ORR (11, 12). However, the influence of jiggling force during orthodontic tooth movement (OTM) is not yet fully understood. Therefore, this study focused on the relationship between ORR and jiggling force. In an in vivo experiment, we investigated the tartrate-resistant acid phosphatase (TRAP) expression in odontoclasts in resorbed roots during experimental jiggling tooth movement in rats.
Materials and Methods

**In vivo** studies

Animals and application of the orthodontic devices

The animal experimental protocol used in this study was approved by the Ethics Committee for Animal Experiments at the Nihon University School of Dentistry at Matsudo (approval No. AP13MD003). A total of 24 eight-week-old male Wistar rats (body weight 350±10 g; Sankyo Labo Service, Tokyo, Japan) were used for experiments.

Animals were anesthetized with pentobarbital sodium (40 mg/kg body weight) for the application of orthodontic devices. Experimental tooth movement was induced using the method described by Matsuda et al. (13), with a Quad Helix-type device (diameter: 0.012 inch, Stainless steel wire; Tomy International, Tokyo, Japan) ligated to the maxillary first molar cleat using a 0.008-inch stainless steel ligature wire (Tomy International, Inc.). The upper first molar was palatally or buccally moved with an appliance at a force of 10
or 50 g (Fig. 1). The experimental period was 21 days. Rats were randomly assigned to four groups: a control group, where rats received no appliances; an optimal force (OF) group, where rats were subjected to a 10 g compression force; a heavy force (HF) group, where rats subjected to a 50 g compression force; and a jiggling force (JF) group, where rats were subjected to 10 g compression on day 7, 10 g tension on day 14 and 10 g compression force on day 21 (control = 6, OF = 6, HF = 6, JF = 6) (Fig. 2).

Tissue preparation

Animals were deeply anesthetized using thiamylal sodium and were transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, after which the maxilla was immediately dissected and immersed in the same fixative for 18 h at 4 °C. Specimens were decalcified in 10% disodium ethylenediaminetetraacetic acid (EDTA, pH 7.4) solution for four weeks, and decalcified specimens were subsequently dehydrated using a graded ethanol series and embedded in paraffin. Each sample was sliced continuously into 4-μm sections in the frontal direction and was prepared for hematoxylin and eosin (HE) and immunohistochemical staining. Animals in the control group did not experience any tooth movement (Fig. 3).

TRAP staining

Tissue sections were deparaffinized, and endogenous peroxidase activity was quenched via incubation in 3% H₂O₂ in methanol for 30 minutes at room temperature. After being washed in Tris-buffered saline (TBS), sections were incubated with polyclonal anti-rabbit tartrate-resistant acid phosphatase (TRAP; Santa Cruz Biotechnology, Inc., CA, USA; working dilution, 1:100) antibodies for 18 h at 4 °C. TRAP was stained using the Histofine Simple Stain MAX-Po (G) and (R) kit (Nichirei, Co., Tokyo, Japan) in accordance with the manufacturer’s protocol. The Histofine Simple Stain MAX-Po (R) kit was used for TRAP. Sections were rinsed with TBS. Final color reactions were induced using the 3,3'-diaminobenzidine tetrahydrochloride substrate reagent, and sections were then counter-stained with hematoxylin. As immunohistochemical controls, several sections were incubated with 0.01 M phosphate-buffered saline (PBS) instead of primary antibody. Negative reactivity was observed in control samples. TRAP-positive cells were counted as multinucleated odontoclasts, which were observed on the surface of the cementum.

Statistical methods

The values in each figure represent means ± standard deviation (SD) for each group. Intergroup comparisons of average values were performed using a Kruskal-Wallis test, followed by Steel-Dwass test, and values of P < 0.05 and P < 0.01 were considered to indicate a significant difference from the corresponding control.
Results

Body weight during experimental period

No significant differences in body weight were observed among rats in the four groups (data not shown).

Histological changes in periodontal tissues during tooth movement (HE staining)

In the control group (0 g) on days 7, 14, and 21 after tooth movement, PDL specimens were composed of relatively dense connective tissue fibers and fibroblasts that regularly ran in a horizontal direction from the root cementum towards the alveolar bone. Blood capillaries were mainly recognized near the alveolar bone in the PDL. The alveolar bone and root surface were relatively smooth, with a few mononuclear and multinucleated osteoclasts, and resorption lacunae were observed on the alveolar bone surface (Figs. 4–a, e, and i).

In the OF group (10 g), the arrangement of fibers and fibroblasts became coarse and irregular, and blood capillaries were compressed on day 7. Resorption lacunae with multinucleated odontoclasts were not observed on the root surface (Fig. 4–b). On day 14, few root resorption lacunae with multinucleated odontoclasts were observed on the surface of the cementum (Fig. 4–f). On day 21, fewer odontoclasts on the cementum were observed in comparison with those on day 14 (Fig. 4–j).

The PDL in the HF group (50 g) was composed of a coarse arrangement of fibers and expanded blood capillaries. Numerous resorption lacunae with multinucleated odontoclasts were observed on the root on day 7 (Fig. 4–c). On day 14, the numbers of root resorption lacunae with multinucleated odontoclasts were increased on the root surface (Fig. 4–g). On day 21, the resorption lacunae with multinucleated odontoclasts were expanded on the root (Fig. 4–k).

In the JF group, the arrangement of fibers and fibroblasts became coarse and irregular, and blood capillaries were compressed on day 7 (compression) (Fig. 4–d). On day 14 (tension), numerous root resorption lacunae with multinucleated odontoclasts were observed on the root surface, similarly to the HF group (Fig. 4–f). On day 21, resorption lacunae with multinucleated odontoclasts had expanded on the root in comparison with those in the HF group (Fig. 4–l).

TRAP immunohistochemical findings

In the control group (0 g) (Figs. 5–a, e, and i) and the OF
group (Figs. 5-b, f, and j) on days 7, 14, and 21, no resorption lacunae with TRAP-positive multinucleated odontoclasts were observed on root surfaces.

In the HF group, few root resorption lacunae with TRAP-positive multinucleated odontoclasts were observed on root surface on day 7 (Fig. 5-c), and the number of TRAP-positive odontoclasts increased on days 14 and 21 (Figs. 5-g and k).

In the JF group on day 7, no resorption lacunae with TRAP-positive multinucleated odontoclasts were observed
on the root surfaces (Fig. 5-d). On day 14, some resorption lacunae with TRAP-positive multinucleated odontoclasts were present on the root surface (Fig. 5-f). On day 21, TRAP-positive multinucleated odontoclasts on the root were increased in comparison with those in the other groups and in the JF group on day 14 (Fig. 5-l).

Fig. 5  Effects of different orthodontic forces on tartrate-resistant acid phosphatase (TRAP)-positive odontoclasts, as determined by immunohistochemical staining (original magnification \( \times 400 \)).

TRAP positivity was observed in odontoclasts (arrow head) on the cementum in the JF (l) and HF (k) group (f), but not in the OF group (j) on day 21. The number of TRAP-positive odontoclasts (arrow head) on the cementum in the JF group was higher than that in the HF and OF groups on day 21 (f). PDL: periodontal ligament, C: cementum and D: dentine; bar = 50 \( \mu \)m. Direction of applied force is indicated by arrow.
Quantitative evaluation showed that the number of TRAP-positive odontoclasts was significantly higher in the JF group on day 21 when compared with the HF (1.5-fold) and OF groups (3.5-fold) (*$P < 0.01$, Fig. 6).

Discussion

All of the present methods, including the application of a 10 g light force, 50 g heavy force, and jiggling force (10 g), produced tooth movement over a period of 21 days in rats. No significant differences in body weight were observed among rats in the four groups (data not shown). Therefore, these apparatuses did not affect rat growth. Gonzales et al. (7) showed that application of a 10 g light force produced significantly larger tooth movement with significantly less root resorption over a period of 28 days when compared to the application of a heavier force in rats. The optimal force for the movement of the rat upper molars may be even less than 10 g, as suggested previously (14). Many investigators have reported that root resorption is aggravated by increasing force magnitudes (3, 4). For example, Gameiro et al. (15) demonstrated osteoclastic resorption of roots on the mesial surfaces of teeth subjected to heavy orthodontic force (50 g). Therefore, the model used in the present study represents a method for inducing efficient tooth movement and root resorption. Meanwhile, no standard method for the “jiggling” movement has been established. We reproduced a “jiggling force” by moving the rat roots of the molar buccopalatally once a week for 21 days in vivo.

In this study, the HE and TRAP staining results of the OF

and HF groups were largely consistent with those of previous studies (8). However, application of the jiggling force increased the resorption lacunae and TRAP-positive multinucleated odontoclasts in comparison with the HF group on day 21 (Figs. 4 and 5). Figure 6 shows that the number of TRAP-positive odontoclasts was significantly elevated in the JF group on day 21 when compared with the HF (1.5-fold) and OF groups (3.5-fold) (Fig. 6).

ORR occurs at the periphery of the necrotic hyalinized tissue (16), and its pattern at the site of compression is related to the lesions (17). The pathogenesis of ORR is associated with the removal of necrotic tissue from the areas of the periodontal ligament that have been compressed by an orthodontic load (18, 19). Previous studies have also shown that ORR is caused by the removal of necrotic hyalinized tissues (20, 21). TRAP staining in a rat tooth movement model highlighted the involvement of TRAP-positive macrophages and multinucleated giant cells in the removal of hyalinized tissue (18). Nakamura et al. (22) reported that there are 1–2 weeks of lag; the polarity of the force after conversion to the pressure side from the tension side does not change immediately in vivo. Therefore, odontoclasts that secrete TRAP are responsible for the resorption of dental root.

Previous studies reported that two to four weeks are required to remove hyalinized tissue (23, 24, 25). Matsuda et al. (13) suggested that “jiggling movement” leads to compression on both the buccal and lingual sides, so the formation of hyalinized tissues may extend to a wide area. Thus, application of opposing forces before the repair of periodontal tissues leads to the formation of hyalinized tissues, consequently aggravating ORR. Taken together with the present results, these findings suggest that a “jiggling force” may induce ORR during OTM, and maybe a risk factor for ORR.

Recent studies have suggested that the OPG/RANKL/ RANK system and interleukins-6, 8, and 17 play an important role in ORR (8, 9, 10, 26, 27). Further studies are necessary to confirm the relationship between these cytokines and jiggling movement in ORR.

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