Jiggling Force Aggravates Orthodontic Root Resorption Via TNF-α during Rat Experimental Tooth Movement

Keiko Tanaka, Masaru Yamaguchi, Takuji Hikida, Tomokazu Yoshino, Jun Kikuta, Mami Shimizu, Momoko Takahashi, and Kazutaka Kasai

Department of Orthodontics, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan

Abstract

Orthodontic root resorption (ORR) is an unavoidable pathological consequence of orthodontic tooth movement. Swinging of the root due to the reciprocating movement of the tooth (jiggling) may exacerbate ORR. However, little is known about the mechanism how jiggling induces ORR. We herein investigated the tumor necrosis factor (TNF)-α expression in odontoclasts in resorbed roots by jiggling force in vivo.

Twenty-four eight-week old male Wistar rats were divided into four groups, a heavy force group (HF: 50 g), optimal force group (OP: 10 g), jiggling force group (JF: compression and tension, repetition: 10 g) and a control group (no appliances: 0 g). The expression levels of the TNF-α protein in odontoclasts in the dental root were determined using an immunohistochemical analysis.

The immunoreactivity for TNF-α in resorbed roots exposed to the jiggling force was stronger than that in the other groups on day 21. The number of TNF-α-positive odontoclasts was significantly increased in the JF group on day 21 compared with the other groups. These results suggest that a jiggling force may aggravate ORR via TNF-α expression during orthodontic tooth movement.

Introduction

Orthodontic root resorption (ORR) is a common complication associated with orthodontic tooth movement. This is an undesirable consequence which can cause permanent loss in the dental structure of the root apex. The pathogenesis of the condition is related to the removal of necrotic tissue from the areas of the periodontal ligament that has been compressed by an orthodontic load (1–3). Furthermore, many studies have reported that a heavy force induced ORR via inflammatory cytokines such as interleukin-6, 8, receptor activator of nuclear factor kappa-B ligand (RANKL) (4, 5, 6). Furthermore, recent studies reported that a heavy force induced ORR via the expression of tumor necrosis factor (TNF)-α from PDL cells (7), that the levels of TNF-α in the gingival crevicular fluid (GCF) in patients with severe root resorption after orthodontic treatment.

An orthodontist encounters ORR when teeth (especially maxillary anterior teeth) are moved by a jiggling force during orthodontic treatment. A "jiggling" force is that which moves the roots of teeth mesiobuccally or buccolingually during orthodontic treatment. It is thought that a jiggling movement may induce ORR (8, 9). Recent study demonstrated that a jiggling movement induced odontoclast formation and aggravation of ORR in response to heavy force during experimental tooth movement in rats (10). However, the influence of jiggling force during orthodontic tooth movement (OTM) is not yet fully understood. Therefore, this study focused on the mechanism of aggregation of ORR by jiggling force. In an in vivo experiment, we investigated the protein expression levels of TNF-α during experimental jiggling tooth movement in rats.
Materials and Methods

Animals and orthodontic device application

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). In total, 50 eight-week-old male Wistar rats (body weight 350±10g; Sankyo Labo Service, Tokyo, Japan) were used for the experiments.

The animals were anesthetized with pentobarbital sodium (40 mg/kg body weight) for application of the orthodontic devices. Experimental tooth movement was induced using the method described by Matsuda et al. (11), with a Quad Helix type device (diameter: 0.012 inch, Stainless steel wire, Tomy International, Tokyo, Japan) ligated to the maxillary first molar cleat with a 0.008 inch stainless steel ligature wire (Tomy International, Inc.,

![Image](https://example.com/image1.png)

Fig. 1 Experimental tooth movement.

Experimental tooth movement was induced with the design of the appliance (diameter: 0.012 inch, Stainless steel wire) ligated to the maxillary first molar cleat by a 0.008 inch stainless steel ligature wire. The upper first molar was moved palatally or buccally using the appliance with a force of 10 or 50 g. The appliances were attached to rats after the activated for each direction.
Tokyo, Japan). The upper first molar was palatally or buccally moved by the appliance with a force of 10 or 50g (Fig.1). The force was measured the number of grams by using a spring scale. The activated appliance was ligated maxillary first molar in the ligature wire. The experimental period was 21 days. The rats were randomly assigned to four groups. Control group was no appliances. Optimal force (OF) group was treated with 10g of compression. Heavy force (HF) group was treated with 50g of compression. Jiggling force (JF) group was treated with 10g compression from day 0 today 7, 10g tension from day 7 today 14, and 10g compression (paratal side of the root) and 10g tension on day 7, 10g compression on day 14 and 10g tension (buccal side of the root) on day 21 (control = 5, OF = 15, HF = 15, JF = 15) (Fig.2).

The rats were randomly assigned to four groups: control group, no appliances; optimal force (OF) group, treatment with 10 g of compression; heavy force (HF) group, treatment with 50 g of compression; and jiggling force (JF) group, 10 g compression on day 7, 10 g tension on day 14 and 10 g compression and 10 g tension on day 7, 10 g compression on day 14 and 10 g tension on day 21.

**Tissue preparation**

We performed the following in vivo experiments, as described by Nakano et al. (4). Each sample was sliced continuously into 4-μm sections in the frontal direction and prepared for hematoxylin and eosin (HE) and immunohistochemical staining. The periodontal tissue in the buccal and palatal portions of the distal palatal root of the left first upper molar was observed. Detailed observations were made in the "A" area (height : 300 μm × width : 225 μm section (A box) of the root direction from the top of the alveolar bone surface on the palatal side) and "B" area (height : 300 μm × width : 225 μm section (B box) under 150 μm the root direction from the bifurcation on the buccal side), the pressure/tension side during tooth movement (11) (Fig. 3). Both areas were counting the positive cells by hand. The control group animals did not experience any tooth movement.

**Immunohistochemistry**

The tissue sections were deparaffinized, and the endo-
nous peroxidase activity was quenched via incubation in 3% H$_2$O$_2$ in methanol for 30 minutes at room temperature. After washing in Tris-Buffered Saline (TBS), the sections were incubated with polyclonal anti-TNF-$\alpha$ antibody (R&D systems, Inc., Minneapolis, MN, USA; working dilution, 1:100) for 18 hours at 4°C. TNF-$\alpha$ was stained using the Histofine Simple Stain MAX-Po kit (Nichirei, Co., Tokyo, Japan) according to the manufacturer’s protocol. The sections were rinsed with TBS and the final color reactions were performed using the 3,3’-diaminobenzidine tetrahydrochloride substrate reagent and aminoethyl carbazole, and the sections were then counter-stained with hematoxylin. For the immunohistochemical controls, several sections were incubated with either nonimmune rabbit IgG or 0.01 M PBS (phosphate buffered saline) instead of the primary antibody. Negative reactivity was observed.

**Statistics**

The values in each figure represent the mean ± standard deviation (S.D.) for each group. The Kruskal Wallis test was used to compare the means of groups with values of p<0.05 and p<0.01, which were considered to indicate significant differences from the corresponding control.

**Results**

**Body weight during the experimental period**

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

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

Regarding the “A” and “B” areas, in the control group (0 g) on days 7, 14 and 21 after tooth movement, the 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, and the root surfaces were relatively smooth (Figs. 4-a, b, c, d, e, f).

In the OF (10 g) group for the “A” and “B” area, the arrangement of the fibers and fibroblasts became coarse and irregular, and blood capillaries were compressed on days 7 and 14. Resorption lacunae with few multinucleated odontoclasts were observed on the palatal root surface (Figs. 4-g, h, j, k). On day 21, root resorption lacunae with a few multinucleated odontoclasts were observed on the root surface (Figs. 4-i, l).

In the HF (50 g) group for the “A” area, root resorption lacunae with multinucleated odontoclasts were identified on...
The root surface on day 7 after application of the orthodontic force (Fig. 4–m). Many resorption lacunae with multinucleated odontoclasts were observed on the root on day 14 (Fig. 4–n). On day 21, root resorption lacunae with multinucleated odontoclasts were most observed on the root surface (Fig. 4–o). Multinucleated odontoclasts on the palatal root surface and root resorption lacunae gradually increased from day 7 through 21. Conversely, in the HF (50 g) group for the A area, resorption lacunae with few multinucleated odontoclasts were observed on the buccal root surface on days 14 and 21 (Figs. 4–p, q, r).

In the JF (10 g) group for the A and B area, the arrangement of the fibers and fibroblasts became coarse and irregular, and blood capillaries were compressed on day 7, as in the OF (10 g) group. Resorption lacunae with few multinucleated odontoclasts were observed on the palatal root surface (Figs. 4–s, v). On day 14, root resorption lacunae were increased in comparison with day 7 (Figs. 4–t, w). On day 21, many root resorption lacunae containing multinucleated odontoclasts were observed in the palatal root (Figs. 4–u, x).

Protein expression levels of TNF-α

In the control group for the A and B areas, TNF-α-positive cells were rarely observed in the PDL tissues through days 7 to 21 (Figs. 5–a, b, c, d, e, f).
In the OF group, few TNF-α-positive cells were observed in the PDL tissues on the root surfaces in the “A” area through day 21 (Figs. 5-g, h, i). Conversely, in the OF (10 g) group for the “B” area, they were rarely observed in the PDL tissues through days 7 to 21 (Figs. 5-j, k, l).

In the HF group, few TNF-α-positive cells were observed in the PDL tissues on day 7 in the roots in the “A” areas and increased on days 14 and 21 (Figs. 5-m, n, o). Conversely, in the “B” area, no TNF-α-positive cells were observed in the PDL tissues on day 7, whereas a few cells were observed on days 14 and 21 (Figs. 5-p, q, r).

In the JF group, no TNF-α-positive cells were observed on day 7, although cells were observed on day 14 and increased on day 21 in both the “A” and “B” areas of the root surface (Figs. 5-s, t, u, v, w, x).

In our quantitative evaluations, the number of TNF-α-positive odontoclasts was found to significantly increase in the HF and JF groupson day 21 versus the control group for both the “A” and “B” areas (p<0.01). Furthermore, the number of them significantly increased in the JF group on day 21 in comparison with the HF group (p<0.01). There were no significant differences in the “A” and “B” areas between the OF group and the control group (Fig. 6).

Discussion
A root of teeth can be moved three-dimensionally during orthodontic treatment. The movement is called “jiggling”, and may induce ORR. However, no standard method for the “jiggling” movement has been established. Recently, Matsuda et al. (11) developed the model of “jiggling” by
moving the rat roots of the molar buccopalatally once a week for 21 days in vivo. Then, we performed this study based on this method.

All of the methods in present study, 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. Previous study reported that the optimum force for the movement of the rat upper molars may be even less than 10 g (12). Furthermore, Gameiro et al. (13) demonstrated osteoclastic resorption of roots of teeth subjected to heavy orthodontic force (50 g). In this study, no significant differences were observed in the body weight of the rats among the four groups (data not shown). Therefore, these apparatuses did not affect the growth of the rats.

In the "A" area in this study, the HE results for the OF and HF groups were largely consistent with those of previous studies (4, 5, 6). In the JF groups, the application of the jiggling force increased resorption lacunae in comparison with the HF group on day 21 (Figs. 4–t, u). In the "B" area, many resorption lacunae were also observed in the JF group on days 14 and 21 (Figs. 4–w, x). Chen and Darendeliler (14) quantified the extent of root resorption under compression (150 g) and tension (150 g) in human teeth undergoing orthodontic tooth movement using volumetry. The volume of root resorption was greater under compression than under tension or both compression and tension.

Furthermore, there was more root resorption under both compression and tension than under tension. This finding supports the present results.

ORR occurs at the periphery of necrotic hyalinized tissue (1), and the pattern at the site of compression is related to the lesions (15). The pathogenesis of ORR is associated with the removal of necrotic tissue from areas of periodontal ligaments compressed by orthodontic loads (2, 3). Previous studies have also shown that ORR is caused by the removal of necrotic hyalinized tissue (16, 17). Previous studies have reported that two to four weeks are required to remove hyalinized tissue (18). The jiggling movement leads to compression on both the buccal and lingual sides; therefore, the formation of hyalinized tissue may extend to a wide area. Hence, the application of opposite direction forces before periodontal tissue repair induces the formation of hyalinized tissue, consequently aggravating ORR. Eross et al. (19) concluded that jiggling forces, applied alternately in different directions with a short interval of reactivation, are critically important for inducing severe root resorption.

The relationship between ORR and TNF-α has been reported using rat tooth movement models. Heavy forces of 50 g induce ORR via TNF-α production (7). This finding supports the results for the OF and HF groups in this study. In our study, at a jiggling force of 10 g (optimal force), TNF-α-positive PDL cells in the "A" areas were increased in the
JF group on day 21 compared with the HF group (50 g) and OF group (10 g). Many TNF-α-positive odontoclasts were also observed in the JF group on the days 14 and 21 in the “B” area (Fig. 5).

Considering the mechanism of enhancement of TNF-α by jiggling forces, in vitro studies applying compression and tension forces to PDL cells may provide clues. Mitsuhashi et al. (20) demonstrated the mRNA expression of TNF-α increased from PDL cells in a time- and magnitude-dependent manner. Interestingly, tension forces also induce TNF-α from PDL cells (21). Therefore, jiggling forces increase TNF-α may more significantly in response to both compression and tension forces than unidirectional forces.

Regarding the differences between the “A” and “B” areas, the number of TNF-α-positive cells was greater in the JF group in “A” than in the JF group in “B” on day 21 (Fig. 6). The PDL tissues in “A” were exposed to compression forces two times and tension forces once, whereas those in “B” were exposed to tension forces two times and compression forces once. Therefore, the jiggling force may increase cytokines more significantly in the “A” than the “B” area.

In conclusion, these results suggest that jiggling forces may induce ORR via the production of TNF-α during orthodontic tooth movement and may be a risk factor for ORR. Orthodontists must seek to avoid applying jiggling forces to teeth as much as possible to reduce the incidence of ORR.

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