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Effects of aging on RANKL and OPG levels in gingival crevicular fluid during orthodontic tooth movement

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Structured Abstract

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Objectives – To compare the levels of the receptor activator of NF*k*B ligand (RANKL) and osteoprotegerin (OPG) in the gingival crevicular fluid (GCF) during orthodontic tooth movement in juvenile and adult patients.

Design – Fifteen juveniles and 15 adults served as subjects. GCF was collected from the distal cervical margins of the experimental and control teeth at 0, 1, 24, and 168 h after application of a retracting force. Enzyme-linked immunosorbent assay kits were used to determine RANKL and OPG levels in the GCF samples.

Results – The amount of tooth movement for juveniles was larger than for adults after 168 h. Further, after 24 h RANKL levels were increased and those of OPG decreased in GCF samples from the compression side during orthodontic tooth movement in both juveniles and adults. The RANKL/OPG ratio in GCF from adult patients was lower than that in the juvenile patient samples.

Conclusion – Our results suggest that the age-related decrease in amount of tooth movement may be related to a decrease in RANKL/OPG ratio in GCF during the early stages of orthodontic tooth movement.

Key words: aging; gingival crevicular fluid; osteoprotegerin; receptor activator of NF*k*B ligand; tooth movement

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Introduction

As the number of adult patients undergoing orthodontic treatment continues to increase, the differences in anatomic structure and response between young and adult periodontium tissue must be considered before

commencing treatment (1). Goz (2) reported that the biological possibilities for tooth movement are decreased to about one-third in adults when compared with children, while Bridges et al. (3) found that the amount and rate of tooth movement were greater in young rats. Further, the responsiveness of bone to various stimuli seems to decrease with age (4) as active bone-forming capacity also decreases with age (5).

The receptor activator of NF*k*B ligand (RANKL) was recently identified as a member of the membrane-associated tumor necrosis factor (TNF) ligand family and shown to be an important regulatory molecule of osteoclastogenesis (6). RANKL is a ligand of osteoprotegerin (OPG) and is expressed in the plasma membranes of osteoblasts/stromal cells (7). Importantly, it induces osteoclast differentiation from hemopoietic precursors and stimulates their bone resorptive activity (8). OPG, a secreted TNF receptor member that functions as a decoy receptor for RANKL, is an inhibitory factor of osteoclastogenesis in competition with the receptor activator of NF*k*B (RANK) (9,10). Ogasawara et al. (11) detected RANKL in osteoblasts and periodontal ligament (PDL) cells during experimental tooth movement. Thus, RANKL and OPG signaling as well as regulation of their expression may play critical roles in bone remodeling during orthodontic tooth movement. The importance of the RANKL/OPG ratio is demonstrated in patients suffering from severe osteolysis where this ratio was significantly increased (12). Crotti et al. (13) showed that higher levels of RANKL protein were expressed in patients with periodontal disease, while another study found that OPG protein levels were significantly lower in periodontal tissues in patients with periodontal disease than in healthy individuals (14). In their study of gingival crevicular fluid (GCF), Vernal et al. (15) demonstrated that the total amount of RANKL in GCF is significantly increased in association with periodontal disease and a recent *in vivo* study found that the ratio of the concentration of RANKL to that of OPG in the GCF was significantly higher in periodontal disease patients than in healthy subjects (16). These results indicate that an imbalance in the RANKL/OPG system may play an important role in the development of periodontal disease.

Biologically active substances, such as cytokines and enzymes, are expressed by cells within the periodontium in response to mechanical stress from orthodontic appliances (17,18). The overall objective of many investigations has been to better understand the mechanisms for converting physical stress to cellular responses that occur during tooth movement. Our laboratory previously reported that the levels of RANKL were significantly higher and those of OPG significantly lower in GCF samples taken around experimental canines in juvenile subjects (19). However, little information is available concerning the differences between juvenile and adult patients in regards to the production of these modulators during orthodontic tooth movement (20). The present study extends our previous study with data from adults.

In the present study, we compared the levels of RANKL and OPG in GCF samples taken from juvenile and adult patients during orthodontic tooth movement.

Materials and methods

Experimental subjects

Informed consent was obtained from all subjects and the study protocol was reviewed by the ethic committee of Nihon University School of Dentistry at Matsudo. Two groups of orthodontic patients took part in this study. One group consisted of 15 juvenile orthodontic patients (seven males, eight females, mean age 15.1 ± 2.8 years) and the other group comprised 15 adult orthodontic patients (six males, nine females, mean age 31 ± 3.6 years). All subjects were in good general health with healthy periodontal tissues; the probing depths were ≤3 mm, and there was no radiographic evidence of periodontal bone loss. Subjects were excluded if they had had antibiotic therapy during the previous 6 months or if they had taken anti-inflammatory drugs during the month preceding the start of the study.

Experimental design

The upper first premolars in all subjects were extracted and edgewise brackets (0.018 inch × 0.025 inch slot; Tomy International Inc., Tokyo, Japan) were placed in both arches. In each subject, one upper canine was designated as the experimental tooth, while the contralateral and opposing canines were used as control teeth. Canine retraction was performed after leveling. Therefore, the period between extraction and force application was about 3 months. The experimental canines were retracted along a 0.018-inch archwire

using an elastomeric chain (Tomy International Inc.), that delivered an initial force of 250 g.

Impressions for study models were also taken at 0, 1, 24 and 168 h after initiation of tooth movement, and the distances between the distal contact point of the experimental canines and the mesial contact point of the second premolars were measured at those time points with an electronic digital caliper (Max-Cal; Japan Micrometer Mfg. Co. Ltd, Tokyo, Japan) to the nearest 0.01 mm. Each distance was measured 10 times at each time point and the average used as the results.

GCF collection

At each time point, the color of the gingivae was recorded, and the plaque was assessed using the Silness and Loe index (21). To avoid contamination of the GCF samples (22), small deposits of plaque were removed with a periodontal probe. Previous studies of GCF have also used samples collected at 0, 1, 24, and 168 h after initiation of tooth movement.

Using the method of Offenbacher et al. (23), GCF samples were collected from both the experimental and control teeth at each time point. The experimental and control teeth were first gently washed with water, then the gingival area was isolated with cotton rolls (to minimize saliva contamination) and gently dried with an air syringe. Paper strips (Periopaper; Harco, Tustin, CA, USA) were carefully inserted 1 mm into the distal gingival crevice around each experimental and control tooth, and allowed to remain *in situ* for 1 min. After a wait of a further minute, a second strip was placed at the same site for the same length of time. Care was taken to avoid mechanical injury to the gingivae. Following GCF collection, the probing depths and attachment levels of the experimental and control teeth were recorded. Any bleeding following probing was also noted.

The volume of GCF on the paper strip was measured with a Periotron 8000 (Harco) that had been calibrated with human serum. GCF collection was standardized so that the experimental and control sites and different subjects could be compared. After collection the paper strips were stored at −30°C.

RANKL and OPG determination

RANKL and OPG were measured using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA). All samples and standards were assayed twice. Data are shown as the concentrations of cytokine in GCF (pg/μl).

Statistical methods

Values were calculated as the mean ± standard deviation, and statistical analysis was performed using the SAS software system (version 8.2; SAS Institute Japan, Tokyo, Japan). A three-way repeated measures analysis of variance was used in each case to capture the main effects of time, orthodontic tooth movement, and age. Tukey's honest significant difference test was used for multiple comparison.

Results

Clinical parameters

In all subjects, plaque accumulation was minimal throughout the study and gingival health was excellent. Further, probing depths remained less than 2 mm at all times throughout the experimental period and there was no gingival bleeding on probing.

The average amount of tooth movement for juveniles (1.23 ± 0.15 mm) was larger than for adults (0.82 ± 0.22 mm) after 168 h ($p < 0.01$), whereas no movement of the control teeth was detected in any of the subjects at any time point.

GCF volume

GCF volume has been shown to be correlated with inflammatory state, thus we compared the mean volumes of GCF taken from both paper strips used at each time point (24). The mean volume of GCF collected from the adult group was significantly lower than that from the juvenile group for both the experimental and control sites ($p < 0.01$) (Table 1).

GCF levels of RANKL and OPG

RANKL was stable over time and comparable with the controls at the experimental sites, except for 24 h, showing significantly elevated levels, both in juveniles and adults (juveniles: control sites, 9.7 ± 2.3 pg/μl; experimental sites, 210.3 ± 27.6 pg/μl; adults: control sites, 8.2 ± 2.6 pg/μl; experimental sites, 140.5 ±

Table 1. **Total volumes in the GCF in juveniles and adult groups during orthodontic tooth movement**

	Control		Experimental	
Time (h)	Juvenile	Adult	Juvenile	Adult
0	0.47 ± 0.05	0.39 ± 0.06*	0.47 ± 0.08	0.38 ± 0.06*
1	0.47 ± 0.06	0.4 ± 0.04*	0.50 ± 0.10	0.39 ± 0.03*
24	0.42 ± 0.05	0.37 ± 0.05*	0.44 ± 0.05	0.38 ± 0.04*
168	0.45 ± 0.07	0.4 ± 0.03*	0.43 ± 0.08	0.39 ± 0.04*

Significant difference from corresponding group in Juvenile (*$p < 0.01$).

25.5 pg/μl). Further, the level of RANKL in the adult group was lower than in the juvenile group ($p < 0.001$), whereas there were no significant differences between the control sites in both juveniles and adults (Fig. 1a).

OPG was stable over time and comparable with the controls, except for 24 h, showing significantly decreased levels at the experimental sites, both in juveniles and adults (juveniles: control sites, 279.8 ± 32.4 pg/μl; experimental sites, 181.2 ± 37.1 pg/μl; adults: control sites, 278.2 ± 22.1 pg/μl; experimental sites, 235.5 ± 39.5 pg/μl). Further, the level of OPG in the adult group was less than that in the juvenile group ($p < 0.01$), whereas there were no significant differences between the experimental and control sites at 0, 1, and 168 h in both groups (Fig. 1b).

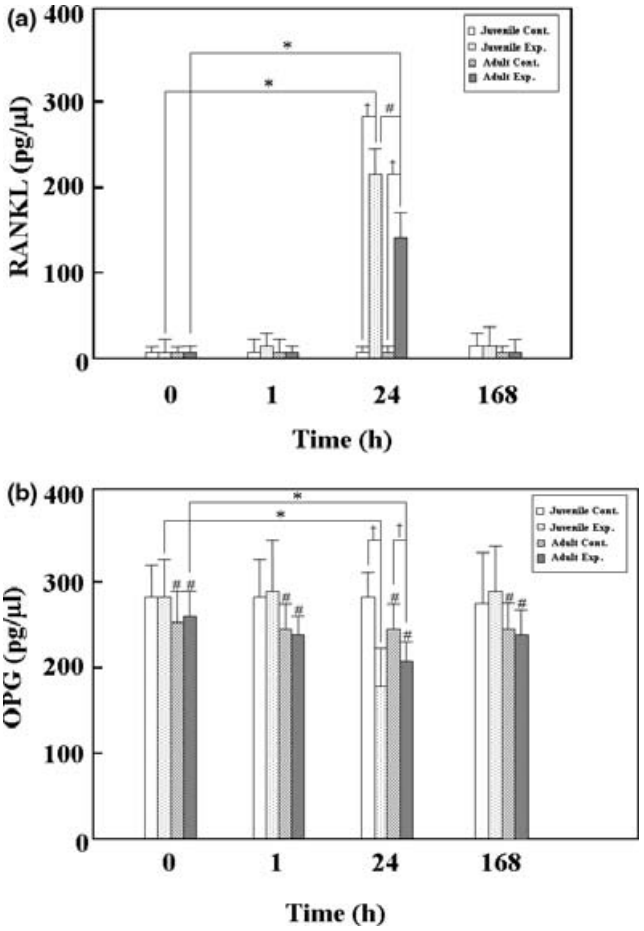

Fig. 1. (a) RANKL and (b) OPG concentrations in the GCF samples from juveniles and adults during orthodontic tooth movement. Contr., contralateral control teeth; Exp., experimental teeth. Significant differences in concentrations between 0 and 24 h are indicated with * ($p < 0.001$), between Contr. and Exp. with † ($p < 0.001$), and between juveniles and adult with # ($p < 0.001$).

RANKL/OPG ratio

The RANKL/OPG ratio for the experimental teeth was significantly higher when compared with the control sites after 24 h in both groups (juveniles: control sites 0.02 ± 0.01; experimental sites 1.17 ± 0.58; adults: control sites 0.03 ± 0.01; experimental sites 0.62 ± 0.034) ($p < 0.001$). Further, the RANKL/OPG ratio for the adult experimental sites was lower than that for the juvenile experimental sites ($p < 0.01$).

Discussion

Some orthodontic procedures require more time when treating adults when compared with juvenile patients, and there are clinical reports of lower anatomic resistance and increased tooth migration in juveniles (25,26). Further, although few experimental studies on the effects of age in regard to orthodontic tooth movement have been performed in rodents, some have indicated that tooth movement occurs at higher rates and over a greater distance in younger rats when compared with adults (1,3,27). However, there is no clinical evidence that adults are less responsive to the mechanical orthodontic stimuli than juveniles.

In the present study, the average amount of tooth movement for juveniles was greater than for adults after 168 h. Ren et al. (28) also reported greater initial tooth movement in juvenile rats. With increasing age, there is a decrease in proliferation of PDL cells, organic matrix production, the relative amount of soluble collagen, and alkaline phosphatase activity (29,30). Further, the bone-formative activity of osteoblasts and bone-resorptive activity of osteoclasts decrease with age (31,32). The mean volume of GCF

collected from the adult group was significant lower than that from the juvenile group for both the experimental and control teeth (Table 1), which agrees with the results of Ren et al. (20), who also reported that GCF volumes in adults were lower than that in juveniles during orthodontic tooth movement. In the present study, the mean RANKL values for the experimental teeth were significantly higher than those for the control sites after 24 h in both groups, although the increase was greater in the juveniles. In addition, the mean OPG values for the experimental teeth were significantly lower than those for the control sites after 24 h in both juveniles and adults, although the decrease was greater in the juvenile group. Also, the ratio of RANKL/OPG was greater in the juvenile group than in the adult group, whereas there was no significant difference between the controls in both juveniles and adult groups.

Periodontal ligament cells may regulate osteoclastogenesis by opposing mechanisms with stimulation of resorptive activity by RANKL and inhibition by OPG (33). RANKL mRNA expression was found to be induced in compressed human PDL cells in a force-dependent manner up to 2.0 g/cm^2, and in a time-dependent manner for up to 48 h (34). Our laboratory also reported that compressive force increased the production of RANKL, and decreased that of OPG in human PDL cells *in vitro* (19). Kanzaki et al. (35) reported that OPG gene transfer to periodontal tissue inhibited RANKL-mediated osteoclastogenesis and inhibited experimental tooth movement. Thus, the signaling and regulation of the expression of RANKL and OPG in PDL may play critical roles in bone remodeling during orthodontic tooth movement. Ren et al. (22) reported that the concentrations of interleukin-6 and granulocyte macrophage-colony stimulating factor in GCF at 24 h were significantly elevated only in juveniles during orthodontic tooth movement, and suggested that the levels of those mediators are more responsive in juveniles than in adults during the early stage of tooth movement. They also reported a faster initial tooth movement in juvenile than in adult rats (33).

Taken together, these findings and our present results suggest that the decrease in amount of tooth movement seen with age may be associated with a decrease in the RANKL/OPG ratio in GCF during the early stages of orthodontic tooth movement.

Conclusions

1. The average amount of tooth movement for juveniles was larger than for adults after 168 h.
2. After 24 h the levels of RANKL were increased and those of OPG decreased in the GCF samples taken from the compression side during orthodontic tooth movement in both juveniles and adults.
3. The RANKL/OPG ratio in the GCF samples from adult patients was lower than that in juvenile patients.

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References

1. Kyomen S, Tanne K. Influences of aging changes in proliferative rate of PDL cells during experimental tooth movement in rats. *Angle Orthod* 1997;**67**:67–72.
2. Goz G. The age dependence of the tissue reaction in tooth movements. *Fortschr Kieferorthop* 1990;**51**:4–7.
3. Bridges T, King G, Mohammed A. The effect of age on tooth movement and mineral density in the alveolar tissues of the rat. *Am J Orthod Dentofacial Orthop* 1988;**93**:245–50.
4. Frost HM. The Utah paradigm of skeletal physiology: an overview of its insights for bone, cartilage and collagenous tissue organs. *J Bone Miner Metab* 2000;**18**:305–16.
5. Jager A. Histomorphometric study of age-related changes in remodelling activity of human desmodontal bone. *J Anat* 1996;**189**:257–64.
6. Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocr Rev* 1999;**20**:345–57.
7. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis - inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A* 1998;**95**:3597–602.
8. Udagawa N, Takahashi N, Jimi E, Matsuzaki K, Tsurukai T, Itoh K et al. Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. *Bone* 1999;**25**:517–23.
9. Burgess TL, Qian Y, Kaufman S, Ring BD, Van G, Capparelli C et al. The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts. *J Cell Biol* 1999;**145**:527–38.
10. Lacey DL, Tan HL, Lu J, Kaufman S, Van G, Qiu W et al. Osteoprotegerin ligand modulates murine osteoclast survival in vitro and in vivo. *Am J Pathol* 2000;**157**:435–48.
11. Ogasawara T, Yoshimine Y, Kiyoshima T, Kobayashi I, Matsuo K, Akamine A, et al. In situ expression of RANKL, RANK, osteoprotegerin and cytokines in osteoclasts of rat periodontal tissue. *J Periodontal Res* 2004;**39**:42–9.

12. Grimaud E, Soubigou L, Couillaud S, Coipeau P, Moreau A, Passuti N et al. Receptor activator of nuclear factor kappaB ligand (RANKL)/osteoprotegerin (OPG) ratio is increased in severe osteolysis. *Am J Pathol* 2003;**163**:2021–31.
13. Crotti T, Smith MD, Hirsch R, Soukoulis S, Weedon H, Capone M et al. Receptor activator NF kappaB ligand (RANKL) and osteoprotegerin (OPG) protein expression in periodontitis. *J Periodontal Res* 2003;**38**:380–7.
14. Liu D, Xu JK, Figliomeni L, Huang L, Pavlos NJ, Rogers M et al. Expression of RANKL and OPG mRNA in periodontal disease: possible involvement in bone destruction. *Int J Mol Med* 2003;**11**:17–21.
15. Vernal R, Chaparro A, Graumann R, Puente J, Valenzuela MA, Gamonal J. Levels of cytokine receptor activator of nuclear factor kappaB ligand in gingival crevicular fluid in untreated chronic periodontitis patients. *J Periodontol* 2004;**75**:1586–91.
16. Mogi M, Otogoto J, Ota N, Togari A. Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. *J Dent Res* 2004;**83**:166–9.
17. Davidovitch Z, Nicolay OR, Ngan PW, Shanfeld JL. Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics. *Dent Clin N Am* 1988;**32**:411–35.
18. Saito M, Saito S, Ngan PW, Shanfeld J, Davidovitch Z. Interleukin-1β and prostaglandin E are involved in the response of periodontal cells to mechanical stress in vivo and in vitro. *Am J Orthod Dentofac Orthop* 1991;**99**:226–40.
19. Nishijima Y, Yamaguchi M, Kojima T, Aihara N, Nakajima R, Kasai K. Levels of RANKL and OPG in gingival crevicular fluid during orthodontic tooth movement and effect of compression force on releases from periodontal ligament cells in vitro. *Orthod Craniofac Res* 2006;**9**:63–70.
20. Ren Y, Maltha JC, Van't Hof MA, Von den Hoff JW, Kuijpers-Jagtman AM, Zhang D. Cytokine levels in crevicular fluid are less responsive to orthodontic force in adults than in juveniles. *J Clin Periodontol* 2002;**29**:757–62.
21. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;**22**:112–35.
22. Griffiths GS, Wilton JM, Curtis MA. Contamination of human gingival crevicular fluid by plaque and saliva. *Arch Oral Biol* 1992;**37**:559–64.
23. Offenbacher S, Odle BM, Van Dyke TE. The use of crevicular fluid prostaglandin E_2 levels as a predictor of periodontal attachment loss. *J Periodontal Res* 1986;**21**:101–12.
24. Cimasoni G. Crevicular fluid updated. In: Myers HM, editor. *Monographs in Oral Sciences*. Basel: Karger; 1983. pp. 1–152.
25. Stepovich ML. A clinical study on closing edentulous spaces in the mandible. *Angle Orthod* 1979;**49**:227–33.
26. Northway WM, Wainright RL, Demirjian A. Effects of premature loss of deciduous molars. *Angle Orthod* 1984;**54**:295–329.
27. Takano-Yamamoto T, Kawakami M, Yamashiro T. Effect of age on the rate of tooth movement in combination with local use of $1,25(OH)_2D_3$ and mechanical force in the rat. *J Dent Res* 1992;**71**:1487–92.
28. Ren Y, Maltha JC, Van't Hof MA, Kuijpers-Jagtman AM. Age effect on orthodontic tooth movement in rats. *J Dent Res* 2003;**82**:38–42.
29. Stahl SS, Tonna EA. H3-proline study of aging periodontal ligament matrix formation: comparison between matrices adjacent to either cemental or bone surfaces. *J Periodontal Res* 1977;**12**:318–22.
30. Van der Velden U. Effect of age on the periodontium. *J Clin Periodontol* 1984;**11**:281–94.
31. Nishimoto SK, Chang CH, Gendler E, Stryker WF, Nimni ME. The effect of aging on bone formation in rats: biochemical and histological evidence for decreased bone formation capacity. *Calcif Tissue Int* 1985;**37**:617–24.
32. King GJ, Keeling SD. Orthodontic bone remodeling in relation to appliance decay. *Angle Orthod* 1995;**65**:129–40.
33. Kanzaki H, Chiba M, Shimizu Y, Mitani H. Dual regulation of osteoclast differentiation by PDL cells through RANKL stimulation and OPG inhibition. *J Dent Res* 2001;**80**:887–91.
34. Kanzaki H, Chiba M, Shimizu Y, Mitani H. PDL cells under mechanical stress induce osteoclastogenesis by receptor activator of nuclear factor *k*B ligand up-regulation via prostaglandin E_2 synthesis. *J Bone Miner Res* 2002;**17**:210–20.
35. Kanzaki H, Chiba M, Takahashi I, Haruyama N, Nishimura M, Mitani H. Local OPG gene transfer to periodontal tissue inhibits orthodontic tooth movement. *J Dent Res* 2004;**83**:920–25.