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Effects of Cortical Activation upon Mechanical Force-Mediated Changes in the OPG and RANKL Levels in Gingival Crevicular Fluid

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This study investigated whether orthodontic force influences the production of osteoprotegerin (OPG) and receptor activator of nuclear factor-kappa B ligand (RANKL) *in vivo*, both of which are affected by cortical activation. Mechanical force was applied to the maxillary premolars of orthodontic patients by fitting the transpalatal arch prior to cortical activation of the gingival tissue. Gingival crevicular fluid (GCF) samples were then collected from each patient using paper strips before and after 1, 3, 7 or 14 days of treatment. The OPG and RANKL levels in the GCF were determined by enzyme-linked immunosorbent assays. The levels of OPG were significantly increased after 1 day of fitting the appliance and decreased to basal levels at 3 days after fitting. In contrast, the RANKL levels were dramatically decreased at 1 day after fitting, but recovered to those of the untreated control at 3 days after the force application. The force-mediated changes in the OPG and RANKL levels of the GCF were unaffected by cortical activation during these experimental periods. Collectively, these results suggest that an acute and severe change between the OPG and RANKL levels plays an important role in stimulating the cellular responses required for alveolar bone remodeling by orthodontic treatment.

Key words: cortical activation; osteoprotegerin, RANKL, gingival crevicular fluid

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Introduction

Application of mechanical force to the teeth makes a biomechanical stimulus to periodontal tissue, causes periodontal ligament (PDL) and alveolar bone remodeling, and eventually leading to tooth movement (Masella *et al*., 2006). Osteoblasts and osteoclasts play crucial roles in regulating the biological events required for the bone remodeling. It is commonly accepted that when mechanical force is subjected, osteoclastogenic activation occurs in the compression side to induce bone resorption, whereas osteoblastic differentiation is stimulated in the tension area (Rygh, 1976). Therefore, the balanced activation between osteoclasts and osteoblasts is critical for alveolar bone remodeling during mechanical treatment.

On the other hand, corticotomy in orthodontic treatment has been applied to facilitate bone remodeling and thus tooth movement. It has been demonstrated that the corticotomy eliminates the mechanical obstacles by cutting off the alveolar bone into 'blocks' (Anholm *et al*., 1986; Gantes *et al*., 1990). It was also reported that this surgical application not only removes the mechanical obstacles, but also promotes bone reformation by inducing a local osteoclastic activation referred as a regional acceleratory phenomena (RAP) (Frost, 1981 & 1989; Yaffe *et al*., 1994). However, this operation is considered to cause several side effects, such as excessive tissue injury, severe bleeding, pain and operational difficulty.

It was recently introduced that cortical activation could reduce the defects derived from corticotomy. This approach is considered to accelerate tooth movement without severe damage to tissue injury (Wilcko *et al*., 2001 & 2003). Cortical activation is also utilized as a mean of stimulating RAP. In addition, it was reported that cortical activation affects the

regeneration of periodontal tissue using animal model (Cho *et al*., 2007). However, the cellular mechanisms by which cortical activation signals cells and/or tissues to facilitate the tooth movement remain unclear.

It has to be noted that the activation of osteoblasts and osteoclasts is tightly affected by a variety of cytokines such as osteoprotegerin (OPG), receptor activator of nuclear factor-kappa B ligand (RANKL), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β and IL-6 (Burgess *et al*., 1999; Ko *et al*., 2008; Lacey *et al*., 2000; Suda *et al*., 1999; Udagawa *et al*., 1999). Among them, OPG and RANKL play the most important role in regulating the activation of these cells; RANKL stimulates osteoclastogenesis, whereas OPG acts as a decoy receptor of RANKL. It has been reported that twist of PDL is occurred after orthodontic force application, and many cytokines are released to gingival crevicular fluid (GCF) at this time (Canlis *et al*., 1991; Davidovitch *et al*., 1988; McColloch *et al*., 1987; Sandy *et al*., 1993). Especially, the levels of RANKL and OPG in the GCF are sensitively changed by mechanical force (Choi *et al*., 2008; Kawasaki *et al*., 2006; Nishijima *et al*., 2006). This means that mechanical force stimulates periodontal cells to produce these cytokines, where the cytokines activate differently osteoclastic or osteoblastic differentiation depending on their ratios secreted. Thus we hypothesized that the facilitation of orthodontic tooth movement by cortical activation is associated with the changes in the levels of OPG and RANKL secreted.

Here we investigated whether the application of mechanical force to tooth actually changes the levels of OPG and RANKL. We also determined how does cortical activation affects the secretion of these cytokines during the application to mechanical force.

Materials and Methods

Application of mechanical force

Five healthy male patients, aged 27.0 ± 1.0 years, were participated in this experiment. Informed written consent from all patients was obtained for use of the GCF before the experiment. This study was also approved by the Ethical Committee of Chonbuk National University Hospital. For application of appliance, edgewise brackets (0.018 × 0.025 inch slot, Tomy International Inc., Tokyo, Japan) were bonded to both maxillary first premolars, and transpalatal arch (TPA) was bent from 0.017 × 0.025 inch TMA wire (Ormco Corp., USA). TPA was tied to these brackets (Fig. 1a). The expansion force of TPA was activated to be 75 g on each side and measured by force gauge.

Cortical activation

After TPA application, cortical activation was applied to the mesiobuccal, distobuccal, mesiolingual, distolingual side of the alveolar bone only in the left maxillary first premolar

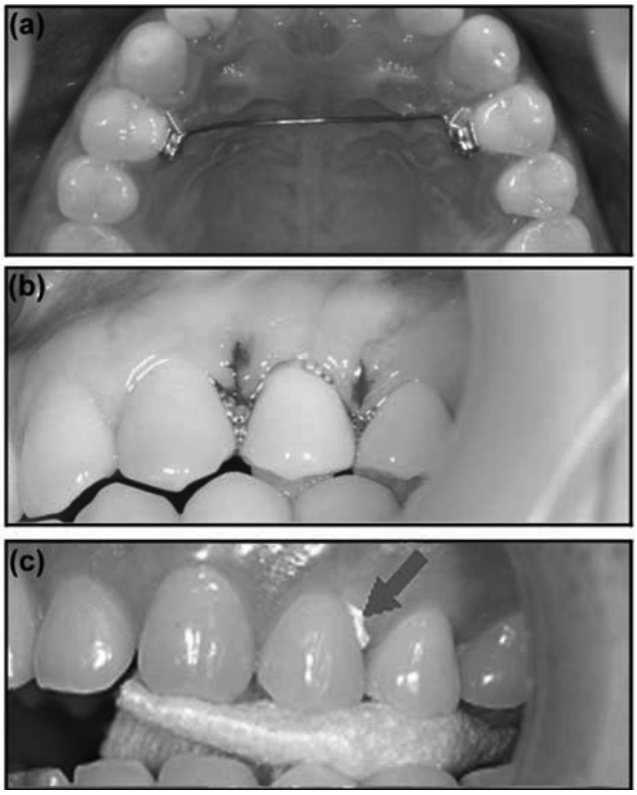

Fig. 1. Experimental designs for mechanical force application, cortical activation and GCF collection. (a) Application of compressive force to maxillary premolar teeth using transpalatal arch and (b) cortical activation to the gingiva. (c) Collection of gingival crevicular fluid (GCF) from the crevicular region using filter paper strips. Arrow indicates the paper strip inserted into the mesiobuccal and distobuccal gingival crevice.

injury as described elsewhere (Wilcko *et al*., 2001 & 2003). Cortical activation was carefully performed with no. 15 mess and hammer (diameter and depth : 5 mm) at a 5 mm interval as to not damage the interproximal papillae (Fig. 1b).

GCF sample preparation

GCF samples were collected from each patient before and after 1, 3, 7 or 14 days of the force application. In brief, deposits of plaque were removed with a periodontal probe before collection of the GCF samples, and the force-applied premolar teeth were washed with distilled water. Filter paper strips were inserted into the mesiobuccal and distobuccal gingival crevice around the force-subjected teeth in a depth of 1 mm for 4 min (Fig. 1c). The patients were given oral hygiene motivation at each appointment after GCF collection. GCF volume per each sample was measured by weighting the strips and then stored at -80°C until before the samples were determined by enzyme-linked immunosorbent assay (ELISA).

Measurement of cytokines

Filter paper strips were resuspended in a 200 μl of phosphate buffered saline (PBS) buffer before centrifugation

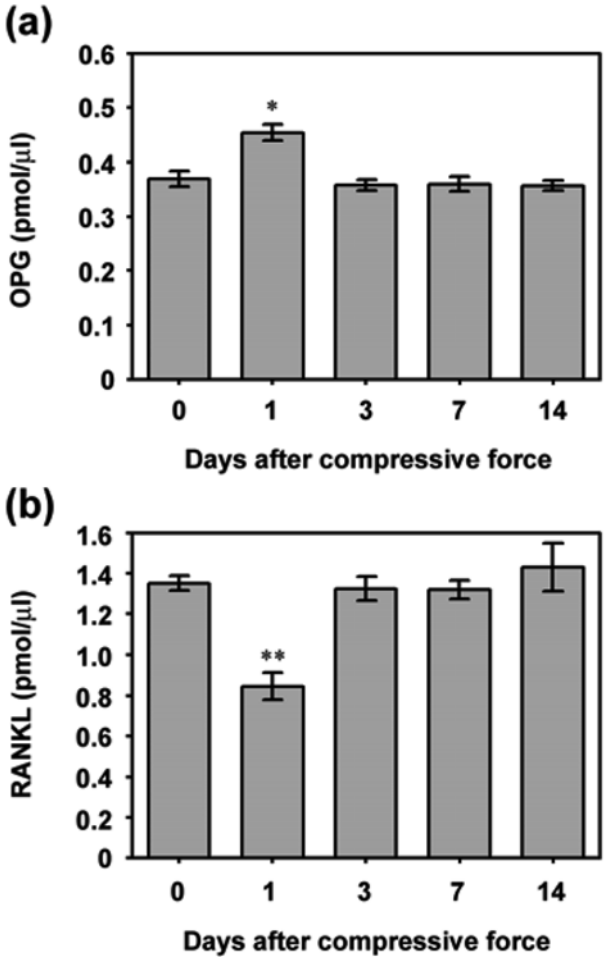

Fig. 2. Effect of compressive force on OPG and RANKL secretion in GCF collected from control site. GCF samples were prepared from the crevicular region of control site using paper strips at the indicated times (0-14 days) and then processed for the analyses of OPG (a) and RANKL levels (b) as described in the materials and methods section. *$p < 0.05$ and **$p < 0.01$ vs. untreated control (day 0).

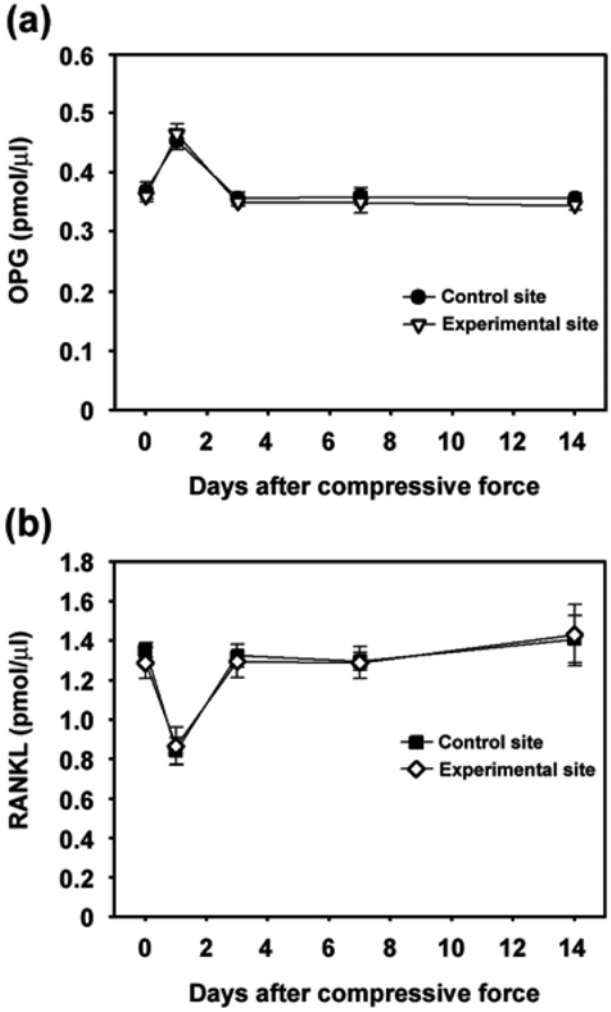

Fig. 3. Effect of cortical activation on the secretion of OPG and RANKL during orthodontic treatment. GCF samples were collected from the crevicular regions of control and experimental sites at various times (0-14 days) using filter paper strips, and the amounts of OPG (a) and RANKL (b) were determined by ELISA.

at 12,000 × g for 10 min. The supernatants were processed for the analyses of OPG and RANKL by ELISA. ELISA kits for human OPG (BI-20402, Biomedica, Vienna, Austria) and RANKL (BI-20422H, Biomedica) were used for the detection of these cytokines, and ELISA was performed according to the manufacturer's instructions. The experiment was performed in duplicate and the data were compared to a standard curve. Finally, the contents of these cytokines were expressed as pmol/μl GCF.

Statistical analysis

All the data are expressed as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) (SPSS version 16.0 software) followed by Scheffe's test was applied to determine multiple comparisons among the groups. Independent sample *t*-test was operated to compare RANKL or OPG values between the control and the cortical activated groups.

Results

Application of mechanical force *in vivo* led to the temporal change in the secretion of OPG and RANKL

In this study, the tooth subjected to TPA only was referred as control, while it combined with cortical activation was served as experimental site. We initially determined whether the amounts of OPG and RANKL in the control sites are changed according to the times subjected to the TPA. Fig. 2a shows that mechanical force affected differently the level of OPG depending on the experimental periods; OPG level was significantly increased up to approximately 1.23-fold ($p < 0.05$) after 1 day of treatment, as compared to the untreated control (0.37 ± 0.014 pmol/μl), but reduced to the basal level after 3 days of the TPA application. When the RANKL level was measured at the same times, there was also a transient and significant change in the secretion of the cytokine. However, the patterns in the force-mediated change

of these cytokines are clearly differed in that the level of RANKL was measured by 0.84 ± 0.067 pmol/μl at 1 day after the TPA treatment while its level was 1.35 ± 0.037 pmol/μl in the GCF collected from the untreated control site (Fig. 2b). This decrease in the RANKL level was recovered to that of untreated control after 3 days of treatment.

Cortical activation did not affect the mechanical force-mediated secretion of OPG and RANKL

We subsequently investigated the effect of cortical activation on the production of OPG and RANKL during the mechanical force application. Unexpectedly, there were not any significant differences in the secretion of these cytokines between the control and experimental sites (Figs. 3a & b). Although the level of RANKL in the experimental site was seen to be higher than the control site after 14 days of treatment, there was no a significant difference (Fig. 3b).

Discussion

Biomechanical force is considered to stimulate cells presented in the PDL to secrete biologically active substances, such as OPG and RANKL. Changes in the levels of these cytokines can be detected in GCF during mechanical tooth movement (Griffiths *et al*., 1998; Nishijma *et al*., 2006). It is the fact that the interaction between RANK and its ligand RANKL triggers the differentiation and activation of osteoclasts (Katagiri *et al*., 2000). In contrast, this interaction is tightly inhibited by OPG. Thus it is to be a common conception that compression side could exhibit up-regulation of RANKL, whereas the predominant induction of OPG occurs in the tension side during orthodontic tooth movement (Garlet *et al*., 2007).

In this study we demonstrate that mechanical force induces a temporal and prominent change in both OPG and RANKL levels in GCF only at the early stages after mechanical force application. However, the levels of these cytokines in GCF were not affected by cortical activation, regardless of the times subjected to mechanical force. Our current data are differed from the finding that compressive force *in vitro* resulted in the up-regulation of RANKL with the attendant decrease of OPG (Nakao *et al*., 2007). We previously showed that centrifugal force to periodontal fibroblasts stimulated the expression of OPG more than RANKL and inhibited osteoclastogenesis in the cells (Kook *et al*., 2009). It was also reported that a persistent and prolonged activation of RANKL and OPG occurs in the compression and tension sides, respectively, during mechanical treatment (Garlet *et al*., 2007; Nakao *et al*., 2007). In this point, we could suggest several hypotheses to explain these different findings. One is that the results could be differed according to the experimental conditions, i.e., *in vitro* and *in vivo*. This is because that unlike *in vitro* system, the data from *in vivo* model are influenced by various parameters. Another possible mechanism is that cellular responses to mechanical force are differed from various conditions such as the magnitude of the force applied, the types of appliance subjected, the age of patients, and the animal models employed. Further, we have to consider a possibility that the GCF contains these cytokines secreted from both the tension and compression sides. This could make a difficulty in determining the precise effect of mechanical force on the production of cytokines according to the force subjected.

Although it is a common phenomenon that compressive force stimulates osteoclastic activation through production of RANKL, and thus RANKL is a predominant cytokine in the compression side, it is also worthy to consider that the cytokine levels in GCF are the sum of the various cellular responses to mechanical force on compression side. Cytokines such as TNF-α, IL1β, M-CSF and IL-6 might participate in the process of bone remodeling by mechanical force. In addition, periodontal tissues consist of various types of cells, gingival fibroblasts, PDL, osteoblasts, and immune cells. This leads to a limitation in identifying what kinds of factors are actually affected directly by mechanical force *in vivo*. It was reported that OPG is highly expressed in both the processes of bone remodeling and inflammation, which is closely related to the local space environment of the tissues (Bachmann *et al*., 1999; Yamaguchi *et al*. 1998). Thus we assume that the change in OPG and RANKL levels in periodontal tissues signals cells and/or tissues to induce biochemical responses required for bone remodeling, although such change occurs in a fast and temporal manner. We also suggest that a promotion of tooth movement by cortical activation is more correlated with a healing mechanism of injured tissues than a direct stimulation of RANKL expression. More detailed experiments will be needed in order to clarify the precise effect of cortical activation on OPG and RANKL.

In conclusion, mechanical force to first premolar teeth by using TPA induces a temporal and significant change in the levels of OPG and RANKL only at the early stage (around 24 h) after the force application, whereas this was not affected by cortical activation.

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