

Root Resorption and RANKL Concentration in Orthodontic Tooth Movement Accompanied by Topical PGE2 Gel Application

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Abstract

The current study sought to determine root resorption and the correlation between root resorption and Receptor Activator Nuclear β Ligand concentration, following orthodontic force accompanied by the application of PGE2 gel. The subjects were 7 male *Macaca fascicularis*. The maxillary right canine was the experimental tooth while the maxillary left canine served as the control. The gel was applied at 0, 2nd and 4th hours, on a weekly basis for 3 weeks. Root resorption was determined by osteoclast count using histological staining with TRAP. A light microscope with 200x enhancement was used. The RANKL concentration in gingival crevicular fluid was measured using ELISA. Root resorption and RANKL concentration were greater in the experimental tooth than in the control tooth, but the difference was not statistically significant ($p > 0.05$). The correlation between root resorption and RANKL concentration was weak. Root resorption always occurred in both the experimental and the control tooth. PGE2 gel application did not make root resorption worse.

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Introduction

The acceleration of tooth movement during orthodontic treatment has been well researched as it can reduce treatment time and to bring greater benefit for patients. It has been reported previously that some methods can stimulate bone remodeling to accelerate orthodontic tooth movement, such as: drug injection,¹ low energy laser irradiation,² and corticotomy.³ Prostaglandin injection in conjunction with orthodontic force application was reported accelerate orthodontic tooth movement by Yamasaki (1984), and Leiker (1995).¹ Seifi,⁴ et al reported similar results, however PGE₂ injections have drawbacks such

as over resorption of the alveolar bone and root surface, as well as discomfort caused by needle injection. An alternative method to overcome those drawbacks has involved using a gel with PGE₂ as an active agent.

Orthodontic treatment induces mechanical stress and evokes some tissue destruction and biochemical responses through inflammatory mechanisms. In sites in which inflammation has occurred, cells may communicate through the interaction of cytokines.⁵ It is important to understand that the inflammatory mechanisms involved in mechanical stress are cellular responses to tooth movement. Yamaguchi,⁶ reported that human periodontal ligaments (PDL) cells were activated by inflammatory factors such as IL-1 β and PGE₂, which may directly stimulate the formation of osteoclasts through RANKL.

RANKL has been identified as a member of the membrane tumor necrosis factor ligand family, and it is an important regulatory molecule of osteoclast formation. RANKL/RANK signaling regulates the formation of multinucleated osteoclasts, induces osteoclast differentiation,

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and stimulates bone resorptive activities to allow tooth movement within alveolar bone according to the direction of force.⁷ The level of RANKL in gingival crevicular fluid is increased during orthodontic tooth movement.^{7,8}

The undesirable effect of orthodontic tooth movement is root resorption. Mechanical forces induced local inflammation which is an essential feature of tooth movement and is the fundamental component of the root resorption process.⁹ A previous study reported that local administration of PGE₂ following orthodontic force resulted in an increased risk of root resorption.⁴ Low et al,⁹ reported that the process of root resorption was influenced by RANK and OPG. Yamaguchi et al,¹⁰ stated that the compressed PDL cells obtained from patients with severe external apical root resorption produced a large amount of RANKL

Thus, RANKL plays an important role during osteoclast formation and root resorption, and it can be evaluated in *Macaca fascicularis* by determining RANKL concentration in GCF. Recently, RANKL was found to be expressed on the surfaces of osteoblasts and stromal cells, while RANKL receptor was found to be expressed on osteoclast precursors and osteoclasts to transduce the RANKL signal.^{7,8}

This study is aimed to determine the effect of a PGE₂ gel as on root resorption and RANKL concentration.

Materials and methods

Seven *Macaca fascicularis*, under the supervision of a veterinarian in the Primate Research Center in the Bogor Agricultural Institute were included in this study. They were male, approximately 5-6 years old and 5-6 kg in weight, and were in good general condition to be studied. A approval from the Animal Care and Use Committee was sought and granted (No 12-B008-IR) in the Primate Research Center – Bogor Agricultural Institute, Indonesia. Prior to the research, all of the *Macaca* were allowed familiarize themselves with the new environment of the Primate Research Center for 30 days. The maxillary first premolars were extracted two weeks prior to the beginning of the experiments. The maxillary right canine was selected as the experimental tooth while maxillary left canine served as the control. All treatments were

performed under general anesthesia with an intramuscular injection of ketamine hydrochloride (7-10 mg/kg BW) and propofol (0,2 – 0,4 kg/hour BW) by syringe pump. All animals received an antibiotic treatment with Ampicilin (30 mg/kg BW) and ketoprofen (5 mg/KG BW) by intramuscular injection.¹¹

PGE₂ gel and control gel application

The PGE₂ gel was prepared just before starting the experiment based on a preliminary study. The control gel was a gel without active PGE₂. One hundred milligrams of PGE₂ gel was applied to the buccal mucosa of the right maxillary canines, and 100 mg of control gel was applied to the buccal mucosa of the left canines. The gel was applied using a cotton bud for 2 minutes with circular movements. Seven maxillary right canines of the *Macaca* served as the experimental teeth, and seven maxillary left canines served as a the control. The gel was applied at 0, 2nd, and 4th hours, every weeks for three weeks (21 days). After 24 hours of PGE₂ gel application, GCF was collected. Figure 1 shows the fixed orthodontic appliance on the *Macaca* maxillary arch.



Figure 1. Orthodontic appliance set in the maxillary arch of the *Macaca*.

Gingival Crevicular Fluid Collection

GCF was collected from both experimental and control teeth within the same period using the method of Offenbacher et al. The first step of the procedure was to wash the experimental and control teeth gently with Aquadest. The teeth were then dried. Next, the gingival area was isolated with a cotton roll to

minimize any saliva contamination. Paper strips (Periopaper, Harco, Tuscin, CA, USA) were inserted with cotton pliers approximately 1 mm into the gingival crevice until the paper strip reached the base of the crevice. Three locations of the gingival crevice were used for GCF collection: distobuccal, proximal, and distopalatal. After waiting for a few minutes, a second set of strips was placed at the same locations for the same length of time. Any bleeding following probing was also noted. Then, the paper strips were placed individually in Eppendorf tube containing 200 µl of PBS and 20 µl of PMFS and the tubes were stored at -80⁰ C. All of these paper strips were subsequently vortexed three times and centrifuged at 2000 g for 4 minutes at 37⁰ C.¹²The paper strips were removed using small pliers. The estimation of the RANKL concentration was determined according to the ELISA laboratory procedure.¹³

RANKL determination

RANKL was measured using an ELISA kit (USCNK System, USA). All samples and standards were analyzed twice.¹¹ Data are reported as the concentration of RANKL in ng/mL.¹³

Histological Preparation

Macaca were euthanized and necropsied and the mesial and distal interdental area of the maxillary right and left canine roots were dissected, and fixed in 10 % neutral buffered formalin to be demineralized with 10%EDTA. The tissue was cut to a thickness of 5 µm and stained using TRAP.¹⁴ Osteoclasts were counted using a light microscope with 200x enhancement.

Statistical analyses

Data were analyzed using Studentt-test. Differences were considered statistically significant when $p < 0.05$. All data were tabulated, and statistical tests were performed using SPSS 18.

Results

Table 1 depicts root resorption according to osteoclast count and the compression areas of the root of the maxillary canine. Table 1also showsthat the means RANKL concentration in the gingivalcrevicular fluid was greater on experimental teeth with PGE₂ gel application, but there was not a significant difference between the experimental group and the control group ($p > 0,05$).

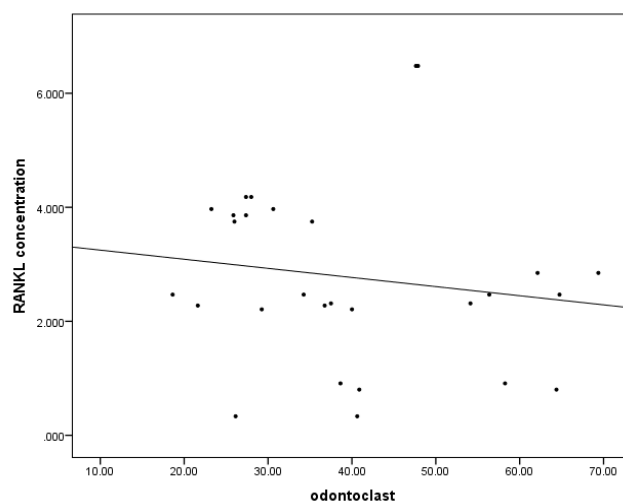


Figure 2. Scatter diagram of RANKL concentration (ng/mL) and osteoclast numbers.

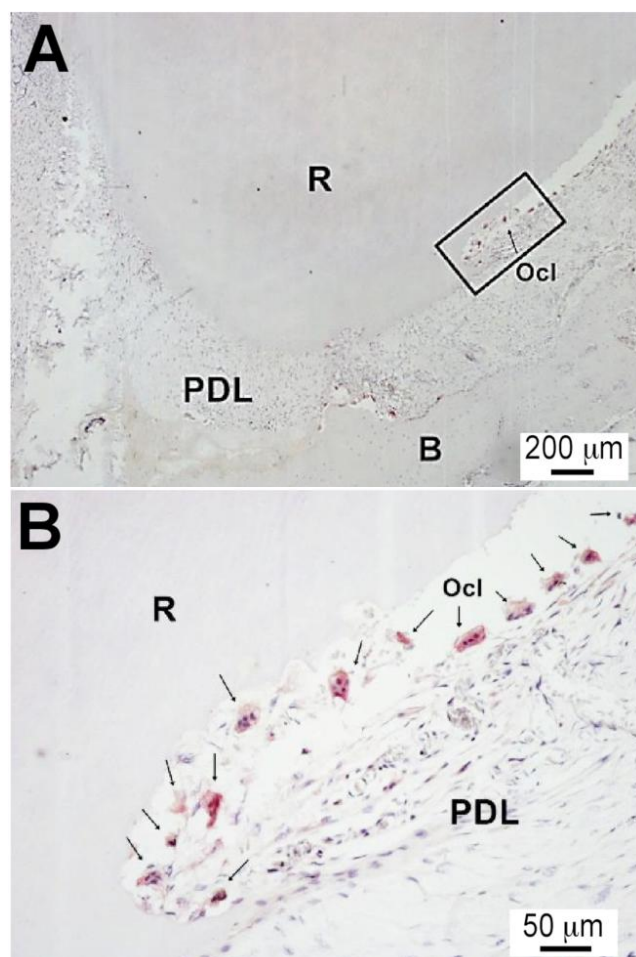


Figure 3. Histological preparation using TRAP staining on the apical experimentaltooth root area (with PGE₂gel). **3A** (left) shows R (tooth root), PDL (periodontal ligament), B (alveolar bone) 40x enhancement. **3B** (right) Oclarrow shows osteoclast, 200x enhancement.

Figure 2 shows the means RANKL concentration in gingival crevicular fluid and the number of osteoclasts. The correlation coefficient was - 0,149.

Histological preparations using TRAP in Figure 3 show that on the root surface there were cavities of varying depth. Some osteoclasts were in groups or formed lines near lacunae on apical root treated with PGE₂ gel.

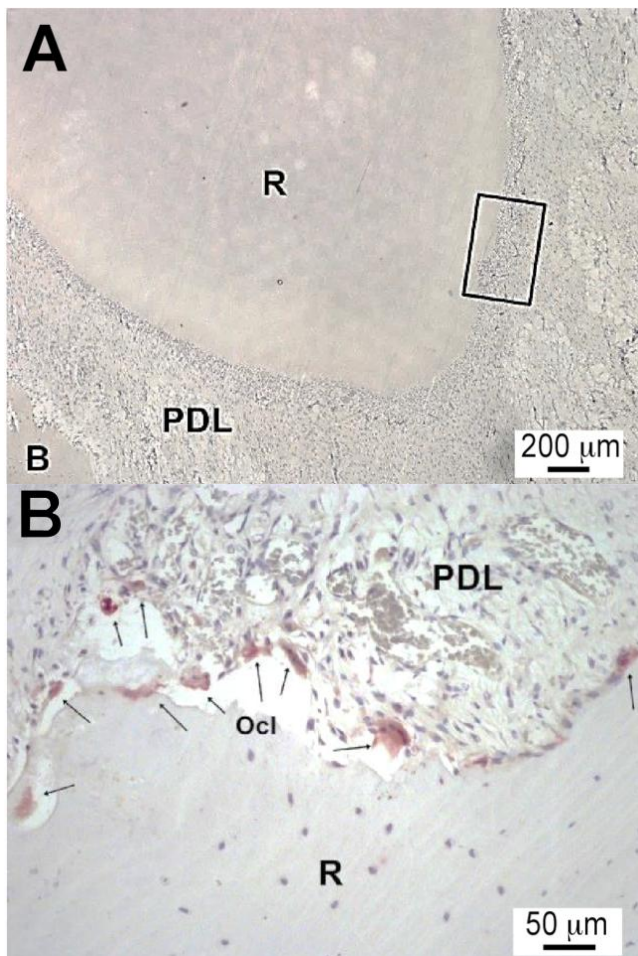


Figure 4. Histological preparation using TRAP staining on the apical root of a tooth treated with control gel (without PGE₂). **4.A** (left) shows R (tooth root), PDL (periodontal ligament), (alveolar bone) 40x enhancement. **4.B** (right) Ocl arrow shows osteoclast 200x enhancement.

Discussion

The injection of prostaglandin combined with orthodontic force can enhance tooth movement.^{1,4} Study prostaglandin for this purpose by injecting PGE₁ locally into the buccal mucosa increasing canine movement by 1.6 to 2

fold compared to untreated teeth. The injection of PGE₂ had been performed in animals at a dosage of 1 – 10 μg/mL within 2-3 weeks (21 days) periodically.¹ Although PGE₂ could enhance tooth movement, there were some side effects such as excessive alveolar bone resorption,^{1,4} root resorption,⁴ and pain.

Based on several studies, it has been shown that PGE₂ may enhance tooth movement. A different form of prostaglandin application was needed to overcome the side effects. One of the forms chosen was a gel due to the general usage of gels on the oral mucosa, such as topical anesthetics and toothpaste.¹⁵ A preliminary study of PGE₂ gel penetration at a dosage of 25 μg/mL had been performed in Sprague Dawley rats at 0, 2, and 4 hours. The study showed that there was a significant increase in the amount of inflammatory cells on the area of PGE₂ gel application compared to the control.¹⁶ The mechanism of inflammation was characterized by PMN cells which stimulate other cytokines such as IL, PGE₂, cAMP, RANK and RANKL, which play a role in the formation of osteoclasts.

It was used 100 g of force to distalize the canine. The force was applied through an open coil spring on both canines using round archwire (.018”), resulting in tipping movement. A compression area occurred on PDL of the distal cervical and apical root areas of the canine. Osteoclasts that formed in the compression area resorbed bone so that the tooth moved. The tooth root also moved inside alveolar bone that underwent resorption.⁵ This study proved that root resorption increased. Table 1 showed that osteoclast numbers were increased in the experimental tooth, although the difference between groups was not statistically significant (p>0,05).

| | Experimental | | Control | | P* |
|------------------|---------------|---------------|---------------|---------------|-------|
| | Range | Mean (SD) | Range | Mean (SD) | |
| Root resorption: | | | | | |
| Osteoclast | 26,13 - 69,38 | 43,95 (14,32) | 26,13 - 69,38 | 35,54 (14,47) | 0.135 |
| RANKL (ng/mL) | 0,34 - 6,48 | 2,81 (1,07) | 0,34 - 6,48 | 2,74 (2,00) | 0.901 |

Independent t test

Table 1. Differences in root resorption and RANKL concentration according to osteoclast numbers in experiment teeth and control teeth in compression areas.

Root resorption occurred in both groups, which meant that the side effect of root resorption due to orthodontic force was inevitable.¹⁷ Nevertheless, PGE₂ gel application on the experimental tooth did not increase root resorption, because the results were statistically similar between the groups. These findings showed that PGE₂ gel is a good a prospective topical medication. Additional research is necessary to determine the effect of the gel on the acceleration of orthodontic tooth movement.

RANKL is an important regulatory molecule involved in osteoclast formation. A previous study using mice showed a positive and strong linear correlation between RANKL concentration and osteoclast numbers.¹⁸ In this study, osteoclast numbers were increased, but RANKL concentration decreased, likely due to the use of ketoprofen in the protocol.¹⁹ As seen in Figure 2 RANKL concentration and osteoclast numbers were irregular and did not have any particular pattern. Therefore, the correlation between root resorption and RANKL concentration was negative and very weak ($r = -0,149$). The differences in our results compared to others is likely due to a reduction in RANKL, the type of animal used and differences in the method of measuring root resorption. Previous studies have measured root resorption using micro computed tomography.¹⁸ Further research regarding the role of other cytokines such as RANK, OPG, and M-CSF in osteoclastogenesis may help explain osteoclast formation. We suggest not using drugs that can affect the concentration of RANKL.

Conclusions

Root resorption and RANKL concentration were not different between teeth undergoing orthodontic force and treated via PGE₂ gel application and teeth treated via control gel application. The correlation between RANKL concentration in gingival crevicular fluid and root resorption was weak and linear.

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Declaration of Interest

The authors report no conflict of interest.

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