The Role of Rankl and Opg in Alveolar Bone Remodeling and Improvement of Orthodontic Tooth Movement Post Coffee Brew Administration

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Abstract

This study was aimed at elucidating the role of RANKL and OPG in the alveolar bone remodeling and improvement of tooth movement induced by orthodontic mechanical force (OMF) post coffee brew administration. 24 rats were divided into 3 groups. Group A: the rats were administered with OMF, group B: OMF + coffee brew at 20mg / 100 g of BW in 2 ml of distilled water, and group C: OMF + caffeine of 1.37 mg / 100 g BW in 2 ml of distilled water. OMF was conducted by ligature wires were applied on permanent maxillary right first molar and both permanent maxillary incisivus. Subsequently, the permanent maxillary right first molar was moved to mesial with closed coil spring. Observations were made on day 15 and day 22 to take GCF and measure the tooth movement on the film resulted from X-ray photograph.

Coffee brew increases the levels of RANKL and OPG, and improves the movement of the teeth, therefore it may be an alternative to accelerate orthodontic treatment.

Keywords: RANKL, OPG, Coffee, alveolar bone remodeling.

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Introduction

Orthodontic tooth movement (OTM) is induced by mechanical stimuli and facilitated by remodeling of the periodontal ligament and alveolar bone. A precondition for these remodeling activities, and ultimately for tooth displacement, is the occurrence of an inflammatory process.

Bone remodeling is a dynamic interaction between bone-forming osteoblasts and bone-resorbing osteoclasts. The rate of remodeling is defined primarily by cells of the osteoblast lineage, which, in addition to bone formation, are also responsible for the activation and recruitment of osteoclast precursors.

Receptor Activator of Nuclear Factor-κB Ligand (RANKL) is a regulator on bone remodeling during orthodontic tooth movement. Receptor Activator of Nuclear Factor-κB (RANK) is a receptor that interacts with RANKL thus triggering the differentiation of osteoclasts which play a role in bone resorption. RANKL is expressed on fibroblasts and osteoblasts in the periodontal ligament in the compression side as well as plays a role in osteoclast differentiation in response to the mechanical force. During the process of alveolar bone resorption, RANKL can also be detected in odontoblasts, osteoclasts, and other cells found in the periodontal ligament.

Osteoprotegerin (OPG) is a glycoprotein produced by human periodontal ligament cells and has been found to be a key factor in the inhibition of osteoclast differentiation and activation. OPG also acts as a receptor for RANKL which competes with RANK to bind and avoid interaction with RANK, thereby occurs inhibition of osteoclastogenesis. Expression of OPG in osteoblasts is regulated by various cytokines, hormones and growth factors and by the Wnt/β-catenin. Pathway of Wnt/β-catenin also regulates bone formation.

Results of studies show that the tooth applied with force 7N for 7 days using Hyrax, rapid expansion equipment, greater RANKL
transcripts was found in the compression side than that in the tension side, and the OPG transcript was found greater in the tension side than that in the compression side. RANKL and OPG were increased in the compression and tension sides compared to the control. Besides administering OPG locally on periodontal tissues can inhibit relapse after orthodontic treatment through inhibition of osteoclastogenesis. Examination using PCR also showed RANKL increased significantly on the compression side in the case group followed by bone resorption caused by orthodontic tooth movement.

Various attempts have been made to accelerate orthodontic tooth movement, i.e. by using drugs, surgical methods and methods of physical and mechanical stimuli. Coffee today becomes one of the most popular beverages in the world which is consumed among people. Robusta coffee contains caffeine, that increases osteoclastogenesis through enhancement of RANKL. The result of the study on the rats applied with orthodontic mechanical force (OMF) shows that a high dose of caffeine administration to the rats increases the amount of osteoclast and bone resorption on the compression side. Robusta coffee also contains Chlorogenic acid and caffeic acid that possess an effect as antioxidant, that may reduce oxidative stress on osteoblast. The results of study show that Chlorogenic acid promotes osteogenesis which is indicated by an increase in bone mineralization.

This study was aimed at elucidating the role of RANKL and OPG in the alveolar bone remodeling and improvement of Orthodontic tooth movement induced by Orthodontic mechanical force post coffee brew administration.

Materials and methods

Type of the study was experimental laboratory. A number of 24 rats (Sprague Dauweley), males, aged 3-4 months, weighed 250-300 gram and stated healthy, were objected to the study. The rats were randomly divided into 3 groups. Group A: the rats were applied with orthodontic mechanical force (OMF), Group B: the rats were applied with OMF and administered with dried coffee brew at 20mg / 100 g BW dissolved in 2 ml of distilled water, and group C as control #: the rats were applied with OMF and administered with pure caffeine (TCI-American-United States) of 1.35 mg / 100 g BW dissolved in 2 ml of distilled water. OMF in rats was conducted by anesthetizing rats using ketamine, subsequently ligature wire with a diameter of 0.20 mm was set on permanent maxillary right first molar and both permanent maxillary incivisus. The permanent maxillary right first molar was moved to the mesial using Tension Gauze to generate power of 10 g / cm2 with a Nickel Titanium Orthodontic closed coil spring of 6 mm long. The observations were carried out on the days 15 and 22 to take the gingival crevicular fluid (GCF) by placing the paper points to gingival sulcus of mesio-palatal permanent maxillary right first molar for 30 seconds and then stored in eppendorf tubes. Increased tooth movement in rats was measured on film of X-ray photograph by measuring from the distal permanent maxillary right second molar to the mesial permanent maxillary right first molar lessened by mesiodistal width of permanent maxillary right first molar and permanent maxillary right second molar using digital calipers. Determination of RANKL and OPG levels was performed by ELISA method.

Analysis of the data was conducted using One-way Anova test followed by Tukey HSD test, Kruskal-Wallis test followed by the Mann-Whitney test, Wilcoxon Signed Ranks test, Paired t-test, and Brown-Forsythe Statistics with 95% level of significance (α = 0.05). This study has been approved by the ethical research committee of the Faculty of Dentistry, Airlangga University, Number: 18/KKEPK.FKG/II/2015.

Results

The results of taking GCF from mice No. 8 in group C were damaged due to poor storage thus only seven data were resulted from the group C. The results of the coffee brew effects on the levels of RANKL and OPG are shown in Table 1 (see the appendix).

The test of RANKL level on the day 15 among the research groups based Kruskal-Wallis test followed by the Mann Whitney test showed that the levels of RANKL in group B was greater than in group A and C (p<0.05). RANKL levels in group C was greater than in group A (p<0.05). OPG assay on the day 15 among the groups using Kruskal-Wallis test subsequently followed by Mann-Whitney test showed that level of OPG in group B was greater than that in the groups A.
and C (p<0.05). OPG level in group C was greater than group A (p>0.05). RANKL level test on day 22 using One-way Anova test followed by the Tukey HSD test results showed that RANKL level of group B was greater than either group A or group C (p<0.05), and group C was greater than the group A (p<0.05). OPG level test on day 22 using One-way Anova test followed by Tukey HSD test results showed that the level of OPG in group B was greater than that in groups A and C (p<0.05). OPG level in group C was greater than that in group A (p<0.05).

### Table 1. Comparison of GCF: RANKL and OPG Levels of All Groups in the Observation of Day 15 and Day 22.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>RANKL (pg/ml)</th>
<th>OPG (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Mean ± Standard Deviation)</td>
<td>(Mean ± Standard Deviation)</td>
</tr>
<tr>
<td>A (Control)</td>
<td>8</td>
<td>17.30 ± 5.93a</td>
<td>10.95 ± 4.16b</td>
</tr>
<tr>
<td>B (Treatment)</td>
<td>8</td>
<td>41.62 ± 6.22a</td>
<td>36.91 ± 4.35a</td>
</tr>
<tr>
<td>C (Control)</td>
<td>7</td>
<td>23.15 ± 6.78a</td>
<td>31.28 ± 3.87a</td>
</tr>
</tbody>
</table>

Notes: *based on One-way Anova, **based on Kruskal-Wallis test, ***The same superscript in one column shows no difference among the groups.

Table 2. Comparison of the Permanent Maxillary Right First Molar Movement of All Groups in the Observation of Day 15 and Day 22.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Tooth Movement (mm) (Mean ± Standard Deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Control)</td>
<td>8</td>
<td>0.67 ± 0.10a</td>
</tr>
<tr>
<td>B (Treatment)</td>
<td>8</td>
<td>0.72 ± 0.10a</td>
</tr>
<tr>
<td>C (Control)</td>
<td>7</td>
<td>0.75 ± 0.10a</td>
</tr>
</tbody>
</table>

Notes: *based on One-way Anova, **based on Brown-Forsythe Statistics, ***based on paired t-test, ****the same superscript in one column indicates no differences among the groups.

The test results based on paired t-test in group A and Wilcoxon Signed Ranks test in group B showed a decrease in the levels of RANKL on day 22 compared to day 15, however it was not significant (p>0.05), while paired t-test in group C showed a significant decrease (p<0.05). This showed that the administration of coffee brew on day 15 increased the levels of RANKL. The test results based on Wilcoxon Signed Ranks test showed increased levels of OPG on day 22 compared to day 15 in group A but they were not significant (p>0.05), whereas Wilcoxon Signed Ranks test in group C showed significantly-increased levels of OPG (p<0.05), and paired t-test in group B showed insignificantly-decreased levels of OPG (p>0.05).

The results of the study on the effect of coffee brew to the increased movement of permanent maxillary right first molar to mesial on the day 15 and 22 is showed in table 2. Table 2 illustrates the results of measurement of the increase in the movement of permanent maxillary right first molar to mesial in the group A, group B and group C on the days 15 and 22 (see the appendix). Further test results using Mann-Whitney test on the day 15 showed the increase of movement of the tooth in the groups B and C was greater than that in the group A (p<0.05). The tooth movement in group B was greater than that in the group C (p>0.05). The test using Mann-Whitney on the day 22 showed that the increase in the movement of in group B and C was greater than that in group A (p<0.05). Increased movement of the tooth in group B was greater than that in group C (p>0.05). This showed that the coffee brew increased the orthodontic tooth movement on the days 15 and 22. Further the test using paired t-test on the increase of tooth movement between day 15 and day 22 in group A, group C and group B showed no significant differences (p>0.05).

**Discussion**

Bone resorption and bone formation are parts of the remodeling process during OTM. Bone is deposited on the alveolar wall on the tension side of the tooth with both heavy and light forces, and newly formed bone spicules follow the orientation of the periodontal fiber bundles. On the compression side, with light forces, alveolar bone is directed resorbed by numerous osteoclasts in Howship's lacunae.

Results of the study showed that on the day 15 and day 22, the administration of coffee brew and pure caffeine increases the levels of either RANKL or OPG greater than that is not administered with coffee brew or pure caffeine. Besides, the administration of coffee brew and pure caffeine also increases OTM. Whereas the increase of OTM in the administration of coffee brew is greater than pure caffeine.

Increased levels of RANKL in the coffee brew or pure caffeine administration are caused by caffeine that binds adenosine receptors, and modulates several other receptors including the glucocorticoid receptor, insulin, estrogen, androgen, vitamin D, cannabinoid, glutamate and adrenergic receptors, which are all expressed in osteoblasts or cell osteoprogenitor and possess important functions during osteoblast movements.
Furthermore Lacey show that Vitamin D receptors stimulates RANKL expression in cells such as osteoblasts and bone marrow-derived stromal cells. RANKL produced by osteoblasts subsequently binds to RANK on the surface of osteoclast precursors and recruits the TRAF6 protein adapter, which causes the activation of NF-kB and translocates to the nucleus. NF-kB increases the expression of c-Fos, and c-Fos interacts with NFATc1 to trigger osteoclastogenic gene transcription. OPG inhibits the initiation process by binding to RANKL. Further in vivo studies in animals also show that caffeine reduces bone mineral density (BMD) in rats with increased osteoclastogenesis. Active Osteoclasts will result in bone resorption.

Caffeine also increases levels of OPG due to increased potential of osteogenic osteoblasts. The results showed that caffeine increases the potential of osteogenic in the baby rat of which mother was administered 50 mg/kg caffeine during pregnancy, thus the increased levels of OPG is caused by the increased potential of osteoblasts that produces OPG. Results of previous studies have shown that administration of low doses of caffeine (0.1 mM) in vitro on primary - derivated adipose stem cells (ADSCs) and bone marrow stromal cells increases osteoblast differentiation through markers ALP, Osteocalcin, OPG, RUNX 2, and Sirtuin 1.

Coffee brew increases OPG levels greater than pure caffeine because coffee contains not only caffeine but also caffeic acid which is a non-phenolic flavonoid acid. Caffeic acid group has an effect as antioxidant that can reduce oxidative stress on osteoblasts. Several studies in vitro and in vivo in animals indicate that oxidative stress reduces the rate of bone formation by decreasing the differentiation and life of osteoblast. In addition, it has been reported that Reactive Oxygen Species (ROS) activates osteoclasts and thus, increases the bone resorption. The antioxidant activity is important to stimulate osteoblastic activity through specific receptors, thus it supports the growth of bone.

Decreased levels of RANKL occurs in the group A, group B and group C on the day 22 if compared to the day 15, despite the decline in group B is not significant. Moreover, on the day 22 group A showed insignificantly-increased levels of OPG. Whereas in group C levels of OPG increase significantly, and in group B levels of OPG decrease insignificantly. This condition is because of the decreased strength of OMF, thus the activity of osteoblasts to produce RANKL also declines. Besides, OPG is a decoy receptor protein which competes with RANK to bind RANKL and inhibits activation of RANKL, thus inhibition osteoklastogenesis occurs.

Increased tooth movement on the administration of coffee brew or pure caffeine due to increased levels of RANKL caused by caffeine, as resulted in this study. Yamaguchi stated that RANKL plays a role in bone resorption, which binds to RANK on osteoclast precursors to activate osteoclasts and leads to bone resorption. The strength of orthodontic pressure will be distributed through the teeth to periodontal ligament and alveolar bone, generate compression side resulting in bone resorption and tension side that will form new bone during tooth movement. The complex molecular signals cause the cellular response for alveolar bone resorption leading to tooth movement. Orthodontic tooth movement will be followed by alveolar bone remodeling and periodontal ligament. Increased OTM on day 22 in coffee brew administration occurred but insignificant compared to day 15. It can be concluded that coffee brew effectively improves OTM on the day 15.

Conclusions

Coffee brew administration increases the levels of RANKL and OPG, and improves OTM thus coffee brew can be an alternative to accelerate orthodontic treatment.

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Declaration of Interest

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