The Gingival Crevicular Fluid Levels of IL-1β, IL-6 and TNF-α in Late Adult Rats

Filiz ACUN KAYA¹, Seher GÜNĐÜZ ARSLAN², Can Ayhan KAYA³, Hüseyin ARSLAN⁴, Orhan HAMAMÇI⁵

¹ Associate Professor, Dicle University, Faculty of Dentistry, Department of Periodontology, Diyarbakır, Turkey.
² Associate Professor, Dicle University, Faculty of Dentistry, Department of Orthodontics, Diyarbakır, Turkey.
³ Dr, PhD, Meat and Fish Corporation, Department of Veterinary Diyarbakır, Turkey.
⁴ Associate Professor, Dicle University, Faculty of Medicine, Department of Orthopedics and Traumatology, Diyarbakır, Turkey.
⁵ Professor, Dicle University Faculty of Dentistry, Department of Orthodontics, Diyarbakır, Turkey.

Key Words
Orthodontic tooth movement, gingival crevicular fluid, IL-1β, IL-6, TNF-α, late adult rat.

Abstract
Aim: To evaluate the levels of interleukin1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) in the samples of gingival crevicular fluid (GCF) taken from the late adult rats during the orthodontic tooth movement and to evaluate the responses to orthodontic treatment.

Methodology: In experiment 19 adult (120 days) Spraque-Dawley rats were used. Approximately 15 g force applying open coil spring was applied actively between the upper incisors of the rats. Before and after the activation on the 3rd and 7th and 10th days GCF samples were taken from the vestibular surfaces of appliance fixed teeth using periopaper®. Then the samples were biochemically analyzed. For the statistical analysis of working days of each cytokines repetitive variance analysis technique was used.

Results: The levels of IL-1β, IL-6 and TNF-α were the highest in the 3rd day and started to decrease on the 7th and 10th days.

Conclusions: The cytokine levels of orthodontic force applied teeth in late adult rats are compatible with the levels of studies in young rats.
(Int Dent Res 2011;1:7-12)

Introduction

Although a tremendous increase in the demand for adult orthodontic therapy was seen in the past decades, our knowledge on the efficiency of adult tooth movement through the alveolar bone in adults is indeed possible by means of treatments modalities based on experiences in adolescent. However, certain treatments seem to be more time-consuming in adult than juvenile patients. This Goz to the conclusion that, in adults, the biological possibilities for tooth movement are decreased to about one-third of those found in children (1).

Orthodontic tooth movement is based on forceinduced periodontal ligament (PDL) and alveolar bone remodeling. Mechanical stimuli exerted on a tooth cause an inflammatory response in the periodontal tissues. Inflammatory mediators are released that trigger the biological processes associated with alveolar bone resorption and apposition (2-4).

It was suggested that the presence of neuroimmune interactions may be of importance in
the initial inflammatory response and regenerative processes of the PDLS that are incident to orthodontic tooth movement (5). An important breakthrough in bone biology was the identification of the role of cytokines in bone remodeling. Cytokines are involved in initiating, amplifying, perpetuating, and resolving inflammatory responses. They are key mediators for tissue damage and play an important role in tooth movement (6).

Cytokines are classified as proinflammatory and anti-inflammatory. Proinflammatory ones are TNF-α, IL-1, interleukin 2 (IL-2), IL-6, and interleukin 8 (IL-8). Anti-inflammatory cytokines are interleukins 4, 10, and 13. The proinflammatory ones are alarm cytokines, inducing vascular dilatation with increased permeability and enhancing inflammatory response (6).

IL-1 is a known cytokine that starts the bone resorption process by taking part in the survival, fusion, and activation of the osteoclasts (7,8) IL-1β, a major physiologic form of IL-1, is mainly secreted by monocytes and partially by macrophages, endothelial cells, fibroblasts, and epidermal cells. This secretion is activated by various stimuli (7). All these studies demonstrate that mechanical stimuli activate inflammatory cytokines (6,9). In a cat model, Davidovitch et al.10 have localized induced levels of IL-1β and TNF-α in the periodontium of teeth undergoing movement.

IL-1β and TNF-α affect bone metabolism directly. At extremely low concentrations, IL-1β and TNF-α have been implicated in the process of bone remodeling through specific receptors on the bone cell population (11-13). Monocytes and macrophages do not constitutively produce IL-1β or TNF-α, but on “activation” they synthesize and release these cytokines (13,14).

IL-6 regulates immune responses in inflammation sites and has an autocrine/paracrine activity that stimulates osteoclast formation and bone resorbing activity of preformed osteoclasts (8,9).

Various researchers demonstrated elevated levels of cytokines in tooth movement (12-14). Lynch et al (15) demonstrated that in the early stages of tooth movement (at 12 and 24 hours) cytokines are mostly seen in the periodontal ligament. All studies to determine the levels of cytokines in gingival crevicular fluid (GCF) evaluated animal and human subjects for short times (11-18) These studies applied distalization forces to the teeth and searched for early responses to the forces.

The aims of this study, to evaluate the levels of IL-1β, IL-6 and TNF-α in the samples of gingival crevicular fluid (GCF) taken from the late adult rats during the orthodontic tooth movement and to evaluate the responses to orthodontic treatment.

Materials and Methods

Animal Procedures

In experiment 19 late adult (120 days) Spraque-Dawley rats were used. Approximately 15 g force applying open coil spring was applied actively between the upper incisors of the rats (Fig. 1).

Figure 1. Coil spring was applied actively between the upper incisors of the rats.

GCF Sampling

GCF sampling was obtained with paper strips (Periopaper, Pro Flow, Amityville, NY) using the method described by Rudin et al. (19) Sampling was performed on the vestibular, mesial and distal sides of the tooth to prevent salivary contamination (Fig. 2).

Sample sites were isolated, and the tooth surfaces were air-dried. Paper strips were placed into the sulcus and, after waiting 30 seconds. Paper strips were stored in sterile tubes at −20°C until the day of the experiment.

Saliva and blood contamination was important; contaminated samples were excluded from the study. GCF sampling was done before all other clinical examinations were performed to prevent an increase in fluid volume. Before examination of the GCF, 1000 μL sterile NaCl (9 mg/mL) was added to paper strips, and the GCF was diluted at 3000 g at +5°C for 20 minutes (20).
Cytokines Analysis

The immunoassay system and the machine used for measuring IL-1β, IL-6 and TNF-α concentrations was Immulite (Diagnostic Products Corp, Los Angeles, Calif). For the manual dilution of rats samples, IL-1β, IL-6 and TNF-α–free non-human buffer matrix was used. The amount of IL-1β, IL-6 and TNF-α detected in each sample was compared with IL-1β, IL-6 and TNF-α standard curve that demonstrates a direct relationship between optical dentistry and cytokine concentration. The total amount of IL-1β, IL-6 and TNF-α was determined in picograms.

**Statistical Evaluation**

For the statistical analysis of working days of each interleukin repetitive variance analysis technique was used.

**Results**

The concentration of all cytokines and comparison between the cytokine sampling periods were shown in Table1.

The baseline levels for the concentration of IL-1β, IL-6 and TNF-α increased on the 3th, but decreased on th 7th and 10th days. The difference between the sampling periods for all cytokines is statistically significant (P<.001).

**Table 1.** The comparision of cytokines according to sampling periods.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Sampling period</th>
<th>X̄</th>
<th>S X</th>
<th>S X</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>Baseline</td>
<td>4.07&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.45</td>
<td>0.34</td>
<td>2.30</td>
<td>7.10</td>
</tr>
<tr>
<td></td>
<td>3rd day</td>
<td>24.25&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.03</td>
<td>0.71</td>
<td>20.10</td>
<td>29.50</td>
</tr>
<tr>
<td></td>
<td>7th day</td>
<td>17.02&lt;sup&gt;C&lt;/sup&gt;</td>
<td>1.52</td>
<td>0.36</td>
<td>15.10</td>
<td>19.50</td>
</tr>
<tr>
<td></td>
<td>10th day</td>
<td>12.87&lt;sup&gt;D&lt;/sup&gt;</td>
<td>1.56</td>
<td>0.37</td>
<td>10.20</td>
<td>15.00</td>
</tr>
<tr>
<td>IL-6</td>
<td>Baseline</td>
<td>7.02&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.04</td>
<td>0.72</td>
<td>2.50</td>
<td>12.30</td>
</tr>
<tr>
<td></td>
<td>3rd day</td>
<td>22.51&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.30</td>
<td>0.31</td>
<td>20.10</td>
<td>25.30</td>
</tr>
<tr>
<td></td>
<td>7th day</td>
<td>15.89&lt;sup&gt;C&lt;/sup&gt;</td>
<td>1.68</td>
<td>0.40</td>
<td>12.10</td>
<td>18.20</td>
</tr>
<tr>
<td></td>
<td>10th day</td>
<td>12.98&lt;sup&gt;D&lt;/sup&gt;</td>
<td>1.49</td>
<td>0.35</td>
<td>10.50</td>
<td>15.50</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Baseline</td>
<td>2.92&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.64</td>
<td>0.39</td>
<td>1.10</td>
<td>7.30</td>
</tr>
<tr>
<td></td>
<td>3rd day</td>
<td>16.47&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.17</td>
<td>0.51</td>
<td>11.10</td>
<td>18.90</td>
</tr>
<tr>
<td></td>
<td>7th day</td>
<td>12.09&lt;sup&gt;C&lt;/sup&gt;</td>
<td>2.52</td>
<td>0.59</td>
<td>8.10</td>
<td>17.10</td>
</tr>
<tr>
<td></td>
<td>10th day</td>
<td>9.91&lt;sup&gt;D&lt;/sup&gt;</td>
<td>1.33</td>
<td>0.31</td>
<td>7.10</td>
<td>12.30</td>
</tr>
</tbody>
</table>

The difference between the 2 mean averages shown with different letter in the same column for the same cytokine is statistically significant (P<.001).

**Discussion**

When an orthodontic force is applied to a tooth for a prolonged period of time, an inflammatory response is initiated. As a result of this, a bone resorption process begins and this process accommodates tooth movement (21).

The appearance of osteoclasts and bone resorption are critical factors that initiate tooth movement (22). Mononuclear osteoprogenitor cells in local tissues require several developmental stages to turn into full functional multinucleated osteoclasts (23). Various cytokines and hormones play an important role in this process (23, 24).

Due to the biological limitations of the adult bone, since it well-known that, during aging, the bone composition changes, its cells become less

Figure 2. GCF sampling was obtained with Periopaper®.
reactive, and its metabolism slows (25, 26). Another possible cause might be the use of inappropriate stimuli, because the biological requirements for inducing optimal tissue responses in young and adult individuals may differ (27). Concerning the age effect on bone activity, there is evidence that bone formative activity of osteoblasts and bone-resorptive activity of osteoblast decrease with age (28, 29), but also, in adults, these cells may recover a highly activated state under orthodontic stimuli (30). This reactivation in adults, however, may take more than in juveniles.

The few experimental studies on age effects on orthodontic tooth movement have been performed in rats. Some of them indicate that tooth movement occurs at higher rates and over a greater distance in young than in adult rats (31—33) while others (26, 34) found similar osteoblastic and osteoclastic activity during orthodontic tooth movement in young and adults rats (30, 34). By thinking the results of these studies; in our study no experimental groups in which young rats took place were performed. We thought that this study plan was much more suitable for the ethics of animal studies. Because of this reason, our findings were compared with the results of the studies performed in young rats.

The testing site in this study was the gingival sulcus, because its access in the oral cavity is easy and it has a continuity with the PDL. In rats GCF studies micropipettes were used (35). But we used paper strips that are used frequently in human studies. The reason of choosing this method was that it was more practical and to understand whether this technique can be used in rats.

The maxillary incisive teeths of all patients were monitored because these teeth are accessible. It has been shown that levels of biochemical markers in the GCF might depend on different collection sites (36, 37). For this reason, the incisives were used as both test and control teeth. The control data, collected at the baselines, were obtained before any force was applied. The continuous eruption of the mandibular incisors was blocked, and the incisors were shortened and abraded to some degree during the experiment.

Iwasaki et al (38) reported that IL-1β levels fluctuated with a 28-day cycle when a continuous orthodontic force was applied. In the early stages of orthodontic force application it has been shown that many PDL cells stain positively for IL-1β.10 Also, Lynch et al (15) reported that in the early stages of tooth movement (12 and 24 hours) many PDL cell types stained positively for IL-1β. Lowney et al (12) demonstrated that TNF-α plays a pivotal part in the assessment of orthodontic tooth movement.

Tzannetou et al (18) used low and high forces to the maxillary molars to expand the palate. Low forces were produced by separator placement and higher forces by a palatal expansion device. They observed high levels of IL-1β levels with both the force levels. Also, Lee et al (39) demonstrated that the mean concentrations of IL-1β increase in the first 24 hours after continuous and interrupted forces. All these studies examined GCF in short time periods as compared with this study. They found that especially in the first 24 hours, cytokine levels increased and then equilibrium is reached, which is higher than the baseline levels.

King et al (40) described an early phase of bone resorption (3—5 days), its reversal (5—7 days), and a late phase (7—14 days) of bone deposition. A similar bone cycle has also been reported in humans (41, 42) but in humans this timing seems to be longer than in rats. In our study the aim was to evaluate the early cytokine levels and because of this the working period was limited to 10 days.

The experimental tooth movement leads to significantly increased recruitment of cells that belong to the mononuclear phagocytic system, and it was suggested that the presence of neuroimmune interactions may be of importance in the initial inflammatory response and regenerative processes of the PDLs that are incident to orthodontic tooth movement (43). The macrophage has the ability to produce cytokines, such as IL-1β and IL-6, the levels of which are known to increase during orthodontic tooth movement (44). IL-1β may act synergistically with TNF-α (45) and be a powerful inducer of IL-6 (46, 47). IL-1β, IL-6 and TNF-α were suggested to stimulate bone resorption and bone-cell replication (48, 49).

In our experiment, the maximal level was detected on day 3 after the application of orthodontic force. The decreased number of IL-1β, IL-6 and TNF-α on days 7 and 10. The spring did not require reactivation during the experiment. This fact may explain the reason that IL-1β, IL-6 and TNF-α levels were decreased at 7 and 10 days.

**Conclusions**

The results of this study support the hypothesis that proinflammatory cytokines play a potent role in bone resorption after the application of orthodontic force. The changes in the cytokine levels supports the results of the studies which state that the young and adult rats have similar osteoblastic and osteoclastic activity during orthodontic movement. Also, in our study it was shown that the periopaper® can be used to obtain GCF in rats.
References


