Saliva Components Reestablish the Basal Production of IL-6 by Mononuclear cells, 72 Hours after Nitinol Archwire Placement: A Preliminary Study

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ABSTRACT

The purpose of the study was to evaluate interleukin-6 production, in saliva-activated mononuclear cell cultures from malocclusion patients, before and after placement of .014 NiTi archwires.

Four patients receiving .014 Nitinol archwire to correct malocclusion participated in this study. Samples of their blood and saliva were collected before and after placement of the apparatus. Mononuclear cells were obtained from the blood using the Ficoll-Paque (1.077 g/ml) density gradient separation method. Mononuclear Cells were activated with saliva from each patient and were cultured in 96-well plates for 72 hours. Samples were collected at 24 hours before apparatus placement, and at 24 hours and 72 hours after placement. IL-6 expression levels in the cell culture supernatants were quantified by ELISA.

An increase in IL-6 levels in the cell culture supernatants was observed 24 hours after placement of the orthodontic apparatus relative to the negative control (p= 0.002) and IL-6 came to basal limits 72 hours after apparatus placement.

IL-6 quantification may be useful as a biomarker to estimate the inflammatory response caused by forces applied during orthodontic treatment and their levels came to basal limits 72 hours after apparatus placement in patients without systemic diseases. The isolation of saliva components involved in such effects is important to study the mechanisms to control the acute inflammation in oral cavity after apparatus placement.

Keywords: Dental movement; IL-6; Inflammation; Orthodontic forces; Saliva

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INTRODUCTION

Cytokines are glycoproteins which are involved in the proliferation and differentiation of cell lines and have been implicated in the pathogenesis of periodontal diseases, in bone remodeling, and in bone response to orthodontic treatment. Neurotransmitters and cytokines have been shown to be associated with the activation of alveolar bone and periodontal cells. The initial dental movement provokes an acute inflammatory reaction with leukocyte migration. Bone is reabsorbed in areas of compression, from the side towards which the forces are directed, and the areas of apposition are on the tension side. When we apply the force necessary for orthodontic movements, we produce histological changes that will cause the induction of certain cytokines which act in this local environment.

Inflammatory cytokines which are involved in bone and periodontal remodeling, have been quantified in the crevicular fluid of patients undergoing orthodontic treatment. IL-6 is expressed by monocytes, macrophages, fibroblasts, activated T cells, odontoblasts and other cells in response to microorganisms and to other cytokines, such as IL-1 and TNF-α. Even though we know that orthodontic movements may lead to the liberation of IL-6, we do not know the circumstances under which this might be an effective and auto-regulated inflammatory response and finally is unknown the effect of saliva components on the local auto-regulation of IL-6 expression.

The objective of the present study was to access the IL-6 production, in saliva-activated mononuclear cell cultures from malocclusion patients, before and after placement of .014 NiTi archwires.

MATERIALS AND METHODS

The participants in this study were 4 patients, of both sexes, from the dental clinic at the Center for Graduate Studies and Research of the Universidad Michoacana de San Nicolás de Hidalgo, in the city of Morelia, Michoacán, Mexico, who were receiving .014 Nitinol archwire to correct malocclusion from January 2007 to April 2007.

Inclusion Criteria

Non-smokers patients with dental crowding between 15 and 25 years of age, two males and two females, (21±2 years old) with no previous orthodontic treatment and good periodontal health, without any kind of infection or systemic illness and not under any type of medical treatment or taking any anti-inflammatory medication during the previous 6 months.

Orthodontic Clinical Procedure

Four patients met the inclusion criteria. For each, a medical history and medical records were obtained, impressions and X-rays were taken, diagnoses and treatment plans were made, and bands with tubes were cemented. The cementing was done with 3M ESPE Ketac-Cem™ autopolymerizing glass ionomer and 3M Transbond™ XT Light Cure Adhesive.

Laboratory Tests

Collecting the Biological Sample

From each of the malocclusion patients, 10 ml of peripheral blood were obtained 24 hours before and 24 and 72 hours after placement of the .014 NiTi wires. The blood drawn was used to obtain and separate mononuclear cells. In addition, 3 ml of saliva were collected in sterile Petri dishes from each of the patients at 24 hours before and 24 and 72 hours after apparatus placement of the .014 NiTi wires.

Collecting Mononuclear Cells

The mononuclear (MN) cells were separated using the Ficoll-Paque (Amersham Biosciences, 1.077 g/ml) density gradient separation method. All cells obtained from 10 ml of the patient’s peripheral blood were placed in a tube with an anticoagulant (EDTA). Mixing carefully, they were passed into a 50 ml conical tube containing 10ml of an RPMI 1640+10% inactivated Fetal Bovine Serum medium solution.(Gigco, BRL, Life technologies, Inc NY, USA) To this mixture was added 10ml of 1:2 Ficoll-Paque solution. The conical tube was then centrifuged at 2500 rpm for 30 minutes at 4°C (Eppendorf 5804 R centrifuge, USA). The MN cells were separated with a Pasteur pipette and passed into another conical tube in which they were washed 2 times with 10 ml of phosphate-buffered saline (PBS) solution (0.015 M potassium phosphate, 0.15 M NaCl, pH 7.4) and centrifuged at 2500 rpm for 10 minutes for later use. The cellular suspension was adjusted to a final concentration of 1x10⁶ cells/ml.
Table 1. IL-6 expression detected at each phase of the study.

<table>
<thead>
<tr>
<th></th>
<th>IL-6 Levels (pg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Group A</td>
</tr>
<tr>
<td></td>
<td>X ± SD</td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>N.D.</td>
</tr>
<tr>
<td>Patient 2</td>
<td>1.522 ± 0.1018</td>
</tr>
<tr>
<td>Patient 3</td>
<td>N.D.</td>
</tr>
<tr>
<td>Patient 4</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D. = Not detectable.

In a hemocytometer (Neubauer chamber), using 0.4% trypan blue, a cellular viability of 95% was considered adequate for carrying out the tests.

Stimulation of Mononuclear Cells Using Saliva Components

MN cells were seeded into sterile 96-well flat-bottom plates (Sarstedt, Inc., Newton, NC 28658, USA) at 2x10⁵ cells/100 µl per well in complete RPMI 1640 medium. Each patient’s mononuclear cells were treated with 20 µl per well of their own saliva, and to each well was added 80 µl of RPMI 1640+10% iFBS as a nutrient source. Saliva was sterilized by filtration using 0.22 µm polyethersulfone membrane Millipore filters, (SIGMA, USA) before their use in cultures. The cells were incubated for 72 hours at 37°C in an incubator (CO₂ incubator, SHEL LAB) under 5% CO₂ atmosphere. All tests were performed in triplicate.

The negative control consisted of mononuclear cells from each of the patients which were not treated with saliva: 2x10⁵ cells/100 µl per well and 100 µl of RPMI 1640+10% iFBS as a nutrient source. The culture supernatant was harvested at 24 hours and 72 hours into Eppendorf tubes.

ELISA plates (Biosource International, Inc., Camarillo, California 93012 USA) were used to quantify the IL-6 present in the culture supernatant. The plates were read using an Organon Teknika Micro-ELISA System strip reader to measure absorbance at 450 nm.

Statistical analysis included calculating standard deviations and performing Student’s pairwise t-test for means comparison using the SPSS 10.0 statistical package and written consent was obtained from all patients included in the study, and the protocol was approved by the institutional ethical committee.

RESULTS

Table 1 shows interleukin 6 concentrations detected in the cell culture supernatants of each patient at different points in the study. An increase in the mean IL-6 of all patients is observed in the cell culture supernatants from 24 hours after apparatus placement (Group C) compared to groups A, B, and D, with the greatest IL-6 expression in patient 4, with a mean and standard deviation (SD) of 4 ± 0.396. Table 2 shows via statistical analysis that IL-6 expression levels increased in the cell culture supernatants from 24 hours after placement of the .014 NiTi archwires (Group C), as contrasted with the negative control P=0.002 (Group A) and 24 hours before apparatus placement P=0.006 (Group B). A decrease in IL-6 expression was also observed in the cell culture supernatants from 72 hours after apparatus placement compared to the cell culture supernatants from 24 hours after apparatus placement P=0.001 and this levels were similar to those founded before apparatus placement (Table 1 and Table 2).

DISCUSSION

IL-6 production by mononuclear cells was determined in malocclusion patients experiencing dental movements caused by .014 Nitinol archwire-induced orthodontic forces. The corresponding inflammatory response was evaluated in cultures of mononuclear cells (T lymphocytes, B lymphocytes, NK cells and monocytes) obtained from each patient’s peripheral blood.

These cells synthesize and release IL-6, and also express IL-6R receptors on their membrane surfaces. IL-6 is an inducer of acute-phase reactions in response to inflammation or injured tissue.
Table 2. Statistical analysis by paired means comparison using Student’s t distribution.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Paired Differences Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed) p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td>A vs. B</td>
<td>-.1477</td>
<td>.2469</td>
<td>-3.541 to 5.867E-02</td>
<td>-1.692</td>
<td>7</td>
<td>.134</td>
</tr>
<tr>
<td>Pair 3</td>
<td>A vs. D</td>
<td>-.4318</td>
<td>1.7848</td>
<td>-1.9239 to 1.0664</td>
<td>-3.592</td>
<td>7</td>
<td>.001</td>
</tr>
<tr>
<td>Pair 4</td>
<td>B vs. C</td>
<td>-2.1480</td>
<td>1.5428</td>
<td>-3.4379 to -1.1261</td>
<td>-3.938</td>
<td>7</td>
<td>.006</td>
</tr>
<tr>
<td>Pair 5</td>
<td>B vs. D</td>
<td>-.2840</td>
<td>1.9624</td>
<td>-1.9264 to 1.3566</td>
<td>-3.409</td>
<td>7</td>
<td>.001</td>
</tr>
<tr>
<td>Pair 6</td>
<td>C vs. D</td>
<td>1.8640</td>
<td>1.0367</td>
<td>2.7307 to 5.086</td>
<td>.9973</td>
<td>7</td>
<td>.366</td>
</tr>
</tbody>
</table>

Although most healthy individuals have undetectable serum levels of IL-6, however in various inflammatory situations it is detected in large quantities; therefore, IL-6 increase might be a sensitive parameter to use in researching inflammatory conditions.

Ren Y and collaborators, in 2002, conducted a comparative study in 43 children (11±0.7 years of age) and 41 adults (24±1.6), who were treated orthodontically with .012 Nitinol wire, and whose crevicular fluid levels of cytokine IL-6 were evaluated using absorbent periodontal strips read by radioimmunoassay to observe the kinetic behavior of IL-6 expression during the inflammation process. The results obtained from this assay indicated that the IL-6 concentration increased significantly, from time 0 to time 24 hrs in the child patients (p<0.01).

Comparing these data with our results, we observed that interleukin 6 behaved similarly during the assay, which we demonstrated by applying Student’s t test to our experimental data. Our study participants were 4 patients, of both sexes, and of 18.33±2.73 years of age. In our results we observed all patients presenting an IL-6 increase in cell culture supernatants 24 hours after orthodontic apparatus placement, as compared to the negative control (p=0.002), and as compared to 24 hours before apparatus placement (p=0.006), with cell culture supernatant expression decreasing 72 hours after orthodontic apparatus placement (p=0.001), near to basal levels. In an extensive review of 23 studies on cytokines in crevicular fluid after orthodontic tooth movement that the most consistent result was a peak of proinflammatory cytokine levels at 24 hours and that associations existed between prostaglandin E(2) (PGE(2)) and interleukin-1beta (IL-1beta) and pain, velocity of tooth movement ant treatment mechanics. Goss et al, 1993 demonstrated that during hepatic regeneration, Kupffer cells IL-1 and IL-6 production were elevated and was controlled in an autoregulatory fashion by elevated Kupffer cells PGE2 production.

In accordance to Ren Y et al, 2007, IL-6 was elevated significantly in the early stage of tooth movement and reached significant levels at 24 hours, the authors conclude that the periodontal system stabilizes at a new physiological homeostasis via the downregulation of early-phase proinflammatory cytokines.

The decrease in IL-6 expression level in cell culture supernatants from 72 hours after orthodontic apparatus placement, suggest that orthodontic forces produce an acute-phase, auto-regulated inflammatory response and we suggest to explore saliva components involved in the auto-regulation of IL-6.

CONCLUSIONS

The dental movements induced by orthodontic treatment produce an acute-phase inflammatory response of IL-6, and that the healthy body regulates this response in a period not longer than 72 hours and their quantification can be used for estimating the inflammatory response caused by the forces applied during orthodontic treatment in malocclusion patients with no other comorbidities.

This leads us to think that there is a local regulation of the IL-6 production by saliva components and this is one of the mechanism implicated in the limitation of the inflammatory response at oral cavity after apparatus placement. Finally we suggest characterization of the saliva constituents involved in such effects due to their importance to explore new targets or drugs to treat the inflammatory response in oral cavity.

REFERENCES

Saliva Components Reestablish the Basal Production of IL-6