Juveniles *versus* adults: differences in PGE_2 levels in the gingival crevicular fluid during orthodontic tooth movement

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Abstract: This study aimed to investigate age-related changes in the biosynthetic capacity of prostaglandin E_2 (PGE_2) in the gingival crevicular fluid (GCF) during one month of orthodontic treatment. Twenty-five juvenile subjects (mean age 13 ± 2.1 years) and 23 adults (mean age 24 ± 2.1 years) were included. GCF was collected immediately before the force application at the baseline, 2, 21 and 28 days, with periopaper inserted into the gingival crevice of the maxillary lateral incisors. The mediator levels were determined with an EIA kit. The results showed that the PGE_2 concentrations were significantly elevated from the baseline to 21 days (129.35 and 198.84 pg/μL, $p = 0.0169$) in juvenile subjects and reduced from 21 to 28 days (198.84 to 112.60 pg/μL, $p = 0.0032$). Adults, however, had no significant changes in the PGE_2 levels. The total amounts of PGE_2 from both groups changed between the baseline to 21 and 21 to 28 days ($p = 0.0119$ and $p = 0.0076$, respectively). The PGE_2 initial and final levels showed significant differences between the juveniles and adults, being higher in adults (baseline: juvenile = 129.35 pg/μL vs. adult = 163.20 pg/μL, $p = 0.0379$; t3: juvenile = 112.60 pg/μL and adult = 175.30 pg/μL, $p = 0.0005$). In conclusion, the results demonstrate the presence of variation in the PGE_2 levels according to age and the orthodontic activation period, which can explain why the speed of orthodontics treatment may be different in adults *vs*. juveniles.

Descriptors: Prostaglandins; Gingival crevicular fluid; Orthodontics, corrective; Tooth movement; Cytokines.

Introduction

In orthodontic tooth movement, mechanical stress appears to evoke biochemical and structural responses in a variety of cell types both *in vivo* and *in vitro*.[1-9] The early phase of orthodontic tooth movement involves an acute inflammatory response characterized by local tissue ischemia, periodontal vasodilatation and the migration of leukocytes through the periodontal ligament capillaries.[1-9] In this process, endothelial cells are activated, and cytokines and chemoattractants are produced that result in leukocyte activation. The interaction between activated endothelial cells and leukocytes plays an important role in the inflammatory process by producing proinflammatory and anti-inflammatory cytokines. These mediators are bioactive molecules that regulate the inflammatory process.[2-9] The mechanism of bone remodeling can also be related to the release of

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inflammatory mediators, such as prostaglandin E_2.[1-4]

Prostaglandin E_2 (PGE_2) and interleukin IL-1β are key mediators involved in periodontal diseases, potent stimulators of bone resorption, and are produced by human periodontal ligament cells in response to mechanical stress. PGE_2 not only mediates inflammatory responses, such as increases in vascular permeability and dilatation, but also can act as a potent stimulator of bone resorption and formation. This dynamic mechanism can be affected according to the concentration of PGE_2.[6-10]

Saito *et al.*[9] showed that periodontal ligament cells respond to mechanical stress (*in vivo* and *in vitro*) by an increased production of PGE_2. Thus, after the application of mechanical forces, cells in the periodontal ligament produce sufficient amounts of mediators to diffuse into the gingival crevicular fluid. Grieve *et al.*[7] showed that PGE_2 and IL-1β were significantly elevated after the initial tooth movement but returned to baseline levels after seven days. Ren *et al.*[11] showed that the concentrations were significantly elevated after 24 hours of activation in juvenile and adult patients, but concluded that the mediator levels in juvenile subjects are more responsive than the levels in adults.

Ren *et al.*[5] showed that IL-8, IL-6, IL-1β and TNF-α play significant roles in the early stage of tooth movement, but not in the linear phase. This study suggests that once the microenvironment of the periodontal ligament is activated by orthodontic force, several key proinflammatory cytokines are produced to trigger a cascade of cellular events.

Although the severity of periodontal disease is known to be affected by the age of the host,[12] the role of aging in orthodontic tooth movement has not been well characterized. The production of PGE_2 in human periodontal ligament cells is increased by mechanical stress; however, the age-related changes in the susceptibility of periodontal ligament cells in response to mechanical stress remain unclear.[13]

Little information is available concerning the production of these mediators during orthodontic tooth movement in different ages and during a period of time around the reactivation of the orthodontic appliances in clinical treatment. The literature is limited regarding the best time to reactivate orthodontic appliances in different ages of periodontal cells, which can affect the severity of inflammation and bone resorption in response to the application of mechanical forces.

The level of PGE_2 in the gingival crevicular fluid can increase during orthodontic tooth movement, and its quantification provides a non-invasive model *in vivo* for investigating the dynamics of mediator production.[1,7,14-16] To investigate age-related changes in the biosynthetic capacity of PGE_2 in the periodontal ligament cells, we examined the effects of *in vivo* aging (juvenile and adults) with mechanical tension on the PGE_2 expression by periodontal ligament cells in the gingival crevicular fluid during the first month of orthodontic treatment.

Material and Methods

Study population

Forty-eight orthodontic patients were included in this study, with 25 juvenile subjects, and 23 adults. The juvenile group included the subjects between the ages of 11 and 17 years old, and the adults were from 21 to 27 years old. These subjects met the following criteria: good general health; no use of anti-inflammatory drugs in two month preceding the study; a lack of antibiotic therapy within the past six months; periodontally healthy, with generalized probing depths ≤ 3 mm and no radiographic evidence of periodontal bone loss; no smoking; and a requirement for buccal/labial tooth movement as part of their orthodontic treatment. The Ethics Committee of Research on Human Beings from Taubaté University approved the study. Informed consent was obtained from each individual.

Experimental design

The maxillary right lateral incisors were chosen as the experimental teeth. Orthodontic brackets (*GAC International*) were placed on the teeth and activated by an orthodontic wire (0.012 Nitinol-*GAC International*). This wire was custom-formed for each patient with varying amounts of buccal/labial offset to produce approximately 0.7 N of force at the baseline as measured with a calibrated orthodontic force gauge (Ortoply, Philadelphia, Pennsylvania, USA). The patients received oral hygiene

instructions during all of this study to insure optimal control of bacterial plaque. The radiographic examination and clinical periodontal assessments, including the gingival index (GI),[17] plaque index (PI),[18] probing depth (PD) and clinical attachment level (CAL) were performed at the baseline and the end of experiment. At the mesio/buccal site of the experimental tooth, gingival crevicular fluid was collected immediately before activation (T0 = baseline) and after 2, 21 and 28 days.

The gingival crevicular fluid collection and PGE_2 determination

The gingival crevicular fluid collection was performed as described by Grieve *et al*.[7] Briefly, the mesio/buccal site of the maxillary right lateral incisor was isolated with cotton rolls and gently dried with an air syringe. A paper strip (Periopaper®, Harco. Tustin, CA, USA) was carefully inserted 1 mm into the gingival crevice and maintained for 30 seconds. Immediately, the periopaper strip was placed in an Eppendorf tube and sealed subsequently. The samples were stored at -70°C until the analysis. To quantify the PGE_2 levels, duplicate samples from each site were evaluated with a competitive enzyme immunoassay (EIA) kit obtained from the Cayman Chemical Co. (Ann Arbor, Michigan, USA). The concentrations of mediator in the samples were calculated according to the reference calibration curves of the standards. The results were expressed as pg/μL.

The PGE_2 levels in the GCF were compared among the different periods of orthodontic tooth movement and age. The comparisons between the mean values of two groups were analyzed by analysis of variance and Student's *t*-tests ($p < 0.05$). The data were evaluated with the Bio Estat 2.0 software.

Results

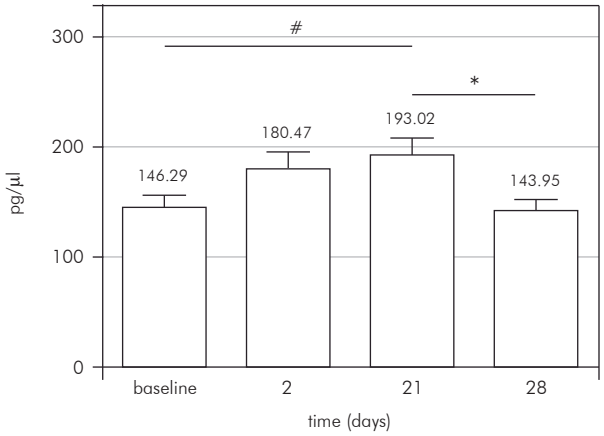
No significant changes in the PI, GI or pocket depth were found in the 48 participants at any time throughout the experiment, suggesting a consistent level of oral hygiene. All of the experimental sites showed a stable and healthy gingival and periodontal status, with the PI and GI lower than 0.25 and pocket depths of 3 mm or less. There were no clinical signs of inflammation at any experimental time point. In relation to the force employed, the mean initial force in the juvenile group was 0.64 N (at the baseline), decreasing to 0.44 N (28 days). In adult group, the initial force was 0.67 N (at baseline), reducing to 0.45 N at the end of experiment. The mean of the CAL and PD and the initial and final force from the juvenile and adult group are shown in Table 1.

The mean levels of PGE_2 in both groups showed significant increases from the baseline to 21 days ($p = 0.0119$) and significant decreases from 21 to 28 days ($p = 0.0076$). However no significant differences were found between the baseline and 28 days ($p = 0.8767$) and 2 to 21 days ($p = 0.5024$). Graph 1 shows that the total PGE_2 levels at the end of experiment (143.95 pg/μL) returned to values similar to the baseline (146.28 pg/μL).

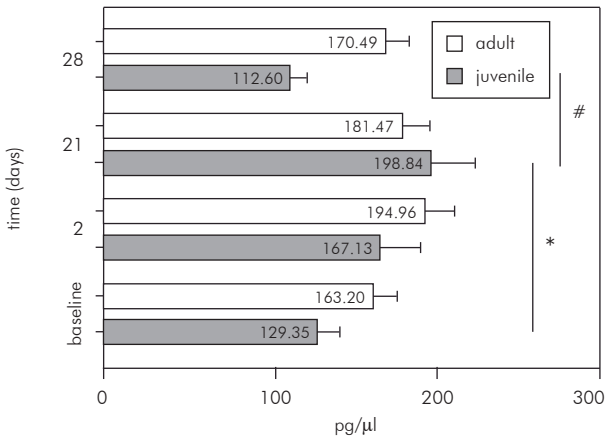
The comparison of the mean concentrations of PGE_2 of the two groups at each time point of study showed significant differences between the total amounts produced by the juveniles and adults at the baseline and the end of experiment. In adults, the amount produced was higher at both time points. At the baseline, the level in adults was 163.20 ± 70.11 pg/μL *versus* 129.35 ± 69.04 in the juveniles ($p = 0.0379$); at 28 days, the levels were 170.49 ± 68.14 pg/μL in adults and 112.60 ± 55.26

Table 1 - The mean ± SD Values for the Age, Probing Depth (PD), Clinical Attachment Level (CAL) and the Initial and Final force in Study Population.

	Juvenile	Adult	Total
N	25	23	48
Female/male	15/10	16/7	31/17
Age	13.60 ± 2.10	24.10 ± 2.10	18.18 ± 5.83
PD (mm)	1.57 ± 0.37	1.52 ± 0.41	1.55 ± 0.38
CAL (mm)	0.87 ± 0.29	0.99 ± 0.30	0.93 ± 0.29
Force (N) initial/final	0.64/0.44	0.67/0.45	0.65/0.44



Graph 1 - Changes in the mean and SEM of PGE_2 in the group total. The levels of PGE_2 in both groups showed significant increases from the baseline to 21 days (p = 0.0119; marked as #), and significant decreases from 21 to 28 days (p = 0.0076; marked as *). The levels did not show significant differences between the other time points.



Graph 2 - Changes in the mean and SEM of PGE_2 in juveniles and adults. In the juvenile group, there was a significant difference between the baseline and 21 days (p = 0.0169; marked as *) and between 21 and 28 days (p = 0.0032; marked as #). In the adult group, the levels of PGE_2 did not show significant changes during the experiment.

in juveniles (p = 0.0005).

In the juvenile group, a significant increase in PGE_2 was observed at 21 days after force had been applied (129.35 pg/µL to 198.84 pg/µL, p = 0.0169). The ANOVA test showed no significant increase between the baseline and 2 days (p = 0.1894) or 2 to 21 days (p = 0.2701). However, a significant decrease (56.6%) occurred at the end of experiment from 21 (198.84 pg/µL) to 28 days (112.60 pg/µL, p = 0.0032) (Graph 2).

Discussion

In orthodontics, mechanical stress evokes a response in a variety of cell types. The early phase of orthodontic tooth movement involves an acute inflammatory response. The gingival crevicular fluid analysis has been proven as an effective method to study periodontal ligament and alveolar bone remodeling.[5] Careful control of bacterially induced inflammation allowed the current research to focus on mediators associated with mechanically induced inflammation within the bone and periodontal ligament. Previous reports have demonstrated reduced or absent quantities of PGE in gingival crevicular fluid at non-inflamed sites.[2] However the increased levels of PGE_2 in gingival crevicular fluid are also associated with an increased severity of periodontal disease.[2,19-20]

Prostaglandins, in particular PGE_2, are involved in the response of bone tissue and cells to stress.[13] In this study, the PGE_2 levels in the initial samples compared to those observed after two days in juveniles did not show a significant difference according to the ANOVA analysis. These data do not agree with previous studies that showed an increase in the levels of PGE_2 in young individuals (17 years on average) after 24 and 48 hours, and a decrease after 168 hours of application of the orthodontic force.[11,21] In another study,[5] in patients 11 to 27 years old, the levels of pro-inflammatory cytokines were significantly elevated in the early stage of tooth movement but at different time points, IL-6, IL-1β and TNFα at 24 hours and IL-8 at 1 month. However, during the linear stage of tooth movement, all cytokines were reduced to their baseline levels. The justification is that the periodontal system stabilizes at a new physiological homeostasis as indicated by the down-regulation of the initial stage of pro-inflammatory cytokines,[5] which can be applied to this study too.

In the juvenile group, there was an increase in PGE_2 from the baseline to 21 days after the application of orthodontic force, while from 21 to 28 days (after a week) there was reduction in the PGE_2 levels.

These data suggest differences in the PGE_2 levels according to the orthodontic movement phase, consistent with the role of PGE_2 as a potent stimulator of bone resorption and formation. This may also suggest that the difference in the PGE_2 activity might be partly affected by the local concentration of PGE_2.

No significant differences were found in the adult group samples at any time. Conversely, some studies showed an elevated PGE_2 production at 24 and 48 hours, but these studies are only 7 days in duration.[7,9,11] The finding of increased levels of gingival crevicular fluid adjacent to teeth undergoing orthodontic tooth movement indicates that the cells within the periodontium are producing increased PGE_2 in response to orthodontic force.

The mean PGE_2 levels of both groups (total group) showed that the total amounts at the end of experiment returned to values similar to those of the baseline, suggesting that the inflammatory process returned to the normal status. This decreased level of PGE_2 suggests that at 28 days there was no more cellular stress, indicating the optimal moment to reactivate the orthodontic appliance.

Lee *et al.* (2004)[22] concluded that while continued strength has been provided, the levels of PGE_2 showed a significant increase in 24 hours and then decreased, showing a temporary increase, and forcefully stopped. The levels of PGE_2 increased significantly in 24 hours and remained high for a week In this study, force is continually applied with the NiTi orthodontic arch, with a gradual increase in the levels of PGE_2, peaking at 21 days then decreasing to levels close to the original.

Several factors could be considered when results are analyzed. First, the analysis of the gingival crevicular fluid is a useful and advantageous method, especially for human *in vivo* studies, because it is noninvasive, and repetitive sampling from the same site is achievable regardless of the number of samples. This enables the monitoring of change at a single site during a certain period.

Comparing the juvenile and adult data, it was possible to observe that juveniles showed greater changes in the PGE_2 levels than the adults, in agreement with Ren *et al.*[11] In the absence of smoking or other periodontal disease, this may explain why the speed of orthodontic treatment may be different in adults *versus* juveniles. This suggests that, in juveniles, the inflammatory responses can react faster to local changes. In juveniles, sex hormones are related to an increased gingival response during orthodontic treatment,[23] however there are no studies that demonstrate the influence of these hormones on the periodontal ligament and alveolar bone in response to mechanical stress. Some studies[11,19,23-24] showed that old periodontal ligament cells produced significantly higher amount of PGE_2 compared with young cells in a time and magnitude-dependent manner when the cells were exposed to a cyclic tension force.

The orthodontic treatment of adults has increased dramatically in recent years. It has been shown that with increasing age there is a decrease in the proliferation of periodontal ligament cells, organic matrix production, the relative amount of soluble collagen and alkaline phosphates activity. Cellular differentiation is also affected, which results in a decreased number of osteoblasts and osteoblast-precursor cells.[11,19,25]

The clinical significance of research regarding the mechanism of bone metabolism in orthodontic tooth movement is related to its potential pharmacologic modulation. This includes the effects of adjunctive PGE and IL-1β, as well as prostaglandin-inhibiting drugs, on the rate of tooth movement.[7]

Conclusions

The analysis of the GCF is an effective method in the study of inflammatory mediators in patients undergoing orthodontic treatment, as well as helpful in periodontal diagnosis. Studies on the mechanisms of the production of inflammatory mediators related to orthodontic therapy at different ages may provide useful information for monitoring the efficiency of tooth movement in the future and enable the establishment of drug regimens to improve the effectiveness of treatment.

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