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Cytokine levels in crevicular fluid are less responsive to orthodontic force in adults than in juveniles

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Abstract

Objectives: Bone remodelling during orthodontic tooth movement is related to the expression of mediators in gingival crevicular fluid (GCF). No information is available concerning the effect of age on the levels of these mediators in GCF. The purpose of this study was to quantify three mediators (prostaglandin E_2, interleukin-6 and granulocyte-Macrophage Colony-Stimulating Factor) in GCF during orthodontic tooth movement in juveniles and adults.

Material and methods: A total of 43 juvenile patients (mean age 11 ± 0.7 year), and 41 adult patients (mean age 24 ± 1.6 year) took part in the study. One of the lateral incisors of each patient was tipped labially, the other served as control. GCF samples were taken before force activation (t_0) and 24 h later (t_{24}). Mediator levels were determined by radioimmunoassay (RIA).

Results: PGE_2 concentrations were significantly elevated at t_{24} in juveniles and adults, while concentrations of IL-6 and GM-CSF were significantly elevated only in juveniles. Total amounts of all three mediators in GCF significantly increased at t_{24} in both groups.

Conclusions: In early tooth movement, mediator levels in juveniles are more responsive than levels in adults, which agrees with the finding that the initial tooth movement in juveniles is faster than in adults and starts without delay.

Key words: adults; cytokines; GCF; juveniles; tooth movement

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Orthodontic treatment in adults has increased spectacularly in the past several decades. In some countries, adults comprise 25% of normal orthodontic practice, and in some big cities, it may be as high as 70% (Norton 1988). The ever-increasing number of adult orthodontic patients calls for a fundamental biological study on modifications in orthodontic approach for this group of patients, indicated by the age-dependent changes in bone remodelling, and subsequent difference in the rate of tooth movement (Kabasawa et al. 1996).

It is a maxim in orthodontics that certain treatments take more time in adult patients than in juveniles. There are clinical reports of lower anatomic resistance and more rapid tooth migration in juveniles than in adults (Stepovich 1979, Northway et al. 1984). Experimental studies in animals indicate that tooth movement is greater and occurs with higher frequency in young than in old individuals (Storey 1955, Bridges et al. 1988). However, there is no clinical evidence that adults are less responsive to the mechanical stimuli than juveniles, once tooth movement has started. The longer treatment period in adults might be caused by a delay in the initial response (Bond 1972, Melsen 1991). There is indirect evidence that the dento-alveolar system undergoes a number of biological changes with age, such as a decrease in bone density, which can affect the responsiveness of periodontal tissues to orthodontic forces (Turner & Spelsberg 1991, Egrise et al. 1992, Liang et al. 1992). It has been shown that with increasing age there is a decrease in proliferation of periodontal ligament cells, organic matrix production, the relative amount of soluble collagen, and alkaline phosphatase activity (Stahl & Tonna 1977, van der Velden 1984). Cellular differentiation is also affected, which results in a decreased number of osteoblasts and osteoblast-precursor cells (Bar-Shira-Maymon et al. 1989, Roholl et al. 1994).

The early phase of orthodontic tooth movement involves an acute inflamma-

tory response characterised by periodontal vasodilation and the migration of leucocytes out of periodontal ligament capillaries. Inflammatory mediators may trigger the biological processes associated with alveolar bone resorption and apposition (Davidovitch et al. 1988). Previous research has suggested that local mediators such as prostaglandins, interleukins and growth factors play an important role in bone remodelling induced by orthodontic forces (Baylink et al. 1993, Vitouladitis et al. 1999, Park et al. 2000). To study these factors in humans, non-invasive methods have been developed using gingival crevicular fluid (GCF) samples (Insoft et al. 1996, Tzannetou & Lamster 1998). The GCF composition is supposed to reflect the physiological status of the periodontal ligament (Last et al. 1988). Grieve et al. (1994) were the first to show that PGE_2 and IL-1β levels in GCF increased significantly after orthodontic force application for 24 and 48 h. Lowney et al. (1995) reported similar effects on TNF-α during tooth movement. Uematsu et al. (1996a, 1996b) found that TGF-β, IL-1β, IL-6, TNF-α and EGF levels are all elevated in human GCF during orthodontic tooth movement. The number of experimental subjects in these studies, however, was relatively low. Furthermore, the difference between juvenile and adult patients has never been studied.

We have chosen to analyse the influence of age on the levels of three mediators in GCF, prostaglandin E_2 (PGE_2), interleukin-6 (IL-6) and granulocyte-macrophage colony stimulating factor (GM-CSF). PGE_2 has long been implicated in bone remodelling and is particularly recognised as a potent stimulator of bone resorption (Offenbacher et al. 1981, Kawaguchi 1995). The role of PGE_2 in orthodontic tooth movement has been the subject of many studies *in vitro*, in animal models and also in humans (Saito et al. 1990a, b, Lasfargues & Saffar 1992, Boekenoogen et al. 1996). IL-6 interacts directly with bone cells. It plays an important role in the local regulation of bone remodelling and in the acute inflammation found at the onset of orthodontic tooth movement (Greenfield 1996). Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) plays a pivotal role in the paracrine regulation of osteoclast and osteoblast differentiation and thereby in bone turnover under physiological conditions (Hattersley 1988, Takahashi 1991). Although little information is available on its production during orthodontic tooth movement, it is likely that application of mechanical forces triggers periodontal ligament cells to produce significant amounts of GM-CSF. To study the biological mechanisms underlying the effect of age on tooth movement, we compared the levels of these three mediators in GCF during initial tooth movement in juvenile and adult patients.

Material and methods

Experimental subjects

Two groups of orthodontic patients took part in the study. One group consisted of 41 adult orthodontic patients (all male, mean age 24 ± 1.6 year) and the other group comprised 43 juvenile orthodontic patients (all male, mean age 11 ± 0.7 year). The study was approved by the Ethics Committee of Research on Human Beings from Beijing Medical University. All patients met the following criteria:

- orthodontic treatment was indicated, and labial movement of the lateral incisors was part of the treatment;
- good general health;
- no antibiotic therapy within the past 6 months;
- no use of anti-inflammatory drugs in the month preceding the study;
- good periodontal health, with generalised probing depths < 3 mm and no radiographic evidence of periodontal bone loss.

Experimental design

The maxillary lateral incisors were chosen as experimental teeth. In one tooth, labial tipping was induced, while the controlateral tooth served as a control. Orthodontic brackets were placed on experimental and control teeth, and the experimental tooth was activated by an orthodontic wire (0.012 Nitinol). This wire was custom made for each patient with varying amounts of buccal/labial offset to produce an initial force of approximately 70 cN. The force was verified with a calibrated orthodontic force gauge. The same appliance, not activated, was placed at the control teeth. The patients were not allowed to take any medication that could affect the production of PGE_2, IL-6 or GM-CSF. To assure optimal control of bacterial plaque, patients received oral hygiene instructions at the start of the study.

GCF was collected before force activation (t_0) and after 24 h of force application (t_{24}) at the experimental and the control teeth.

GCF collection

GCF sampling was performed in an air-conditioned clinic maintained at approximately 22 °C. The sites under study were isolated with cotton rolls. Supragingival plaque was removed, and the region was flushed with water and gently dried with air. Two paper strips (Periopaper, Harco, Tustin, CA, USA.) were carefully inserted 1 mm into the gingival crevice at the mesiobuccal and distobuccal sides of the maxillary lateral incisors for 30 s.

Fluid volumes of all samples were immediately measured with a Periotron 6000® (Siemens Medical Systems, Inc. Iselin, NJ. USA) that had been calibrated with standard volumes of human serum. Immediately after measurement in the Periotron, the periopaper strips from the individual sites were placed in Eppendorf tubes and sealed subsequently. The samples were then stored at -80 °C until analysis. One sample of each tooth was analysed for PGE_2; the other sample from the same tooth was analysed for IL-6 and GM-CSF.

PGE_2, IL-6, and GM-CSF determination

The GCF was eluted from the paper strips by centrifugation with 50-μL aliquots of radioimmunoassay (RIA) buffer. To quantify the levels of PGE_2, IL-6 and GM-CSF, samples from each tooth were evaluated with radio-immunoassay (Advanced magnetics, Cambridge, MA, USA). In short, the assays are based on a competition between mediator in the samples and a standard amount of radiolabelled mediator, which was added. The binding of radiolabelled mediator to the antibody depends on the amount of mediator in the sample. The antibody-bound labelled mediators were quantified in a gamma counter. The concentrations of mediator in the samples were calculated using a standard curve. Total amounts of PGE_2, IL-6, and GM-CSF in each GCF sample were calculated from its total volume.

Statistical analysis

The data on GCF-volumes (in μL), total amounts of PGE_2, IL-6, and GM-CSF (in pg/sample) and their concentrations (in pg/μL) for control and experimental samples at both time points did not appear to be normally distributed, so their median and quartiles were calculated.

All missing data were given the lowest value of baseline samples. After log transformation, the average skewness decreased from 1.85 to 0.22, which allowed the use of *t*-tests for statistical analysis. Two-tailed paired *t*-tests were performed on the transformed data to analyse differences between t_0 and t_{24}, and independent *t*-tests between juveniles and adults for the different parameters. Since the data are skewly distributed, medians are reported instead of means.

Results

The volumes and the concentrations or total amounts of the three mediators in control samples at t_0 and t_{24}, and at t_0 in experimental samples were not significantly different. Therefore, these data were pooled, and the pooled data were used as baseline values for the experimental data. The results are summarised in Figs 1–3 for each of the three cytokines.

The median baseline of GCF volumes in juveniles is 0.17 μL and is higher than the median level of 0.1 μL in adults ($p < 0.01$) (see Figs 1a, 2a and 3a). The median GCF volume in adults increased significantly from 0.1 μL at t_0 to 0.13 μL at t_{24} ($p < 0.05$), while the change from 0.17 μL to 0.2 μL in juveniles was not significant. Median concentrations of all three mediators of GCF in adults at baseline were higher than in juveniles ($p < 0.01$)(see Figs 1b, 2b and 3b). The total amounts of mediators show no differences between juveniles and adults at baseline (see Figs 1c, 2c and 3c).

The median concentrations of all three mediators increased significantly from t_0 to t_{24} in juveniles ($p < 0.01$). The concentration of PGE_2 increased from 252 pg/μL to 458 pg/μL (Fig. 1b); The concentration of IL-6 increased from 64 pg/μL to 76 pg/μL (Fig. 2b). The concentration of CSF increased from 28 pg/μL to 36 pg/μL (Fig. 3b). In adults, only PGE_2 showed a significant increase, from 565 pg/μL to 633 pg/μL ($p < 0.05$). The total amounts of mediators increased significantly from t_0 to t_{24} both in juveniles and in adults (PGE_2 and GM-CSF: $p < 0.01$, Figs 1c and 3c; IL-6: $p < 0.05$, Fig. 2c).

Discussion

This study compared the effect of short-term orthodontic force application on the levels of three mediators in GCF from juveniles and adults. In previous studies of factors in GCF either the concentration or the total amount was reported. According to Lamster (1997) the total amount of a mediator will give more sensitive detection of site-to-site and patient-to-patient GCF differences. Their data, however, show similar results for total amount (pg) and concentration (pg/μL)

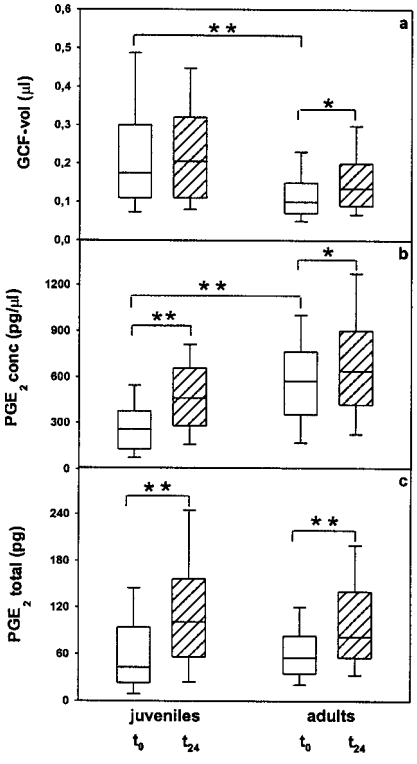

Fig. 1. Boxplots of PGE_2 levels in GCF samples in juveniles and adults from t_0 and t_{24} (median and quartiles).*a*: GCF volume; *b*: PGE_2 concentration; *c*: PGE_2 total amount.

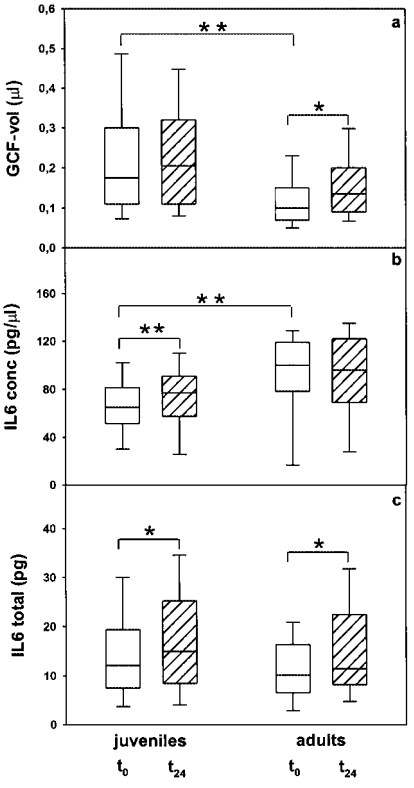

Fig. 2. Boxplots of IL-6 levels in GCF samples in juveniles and adults from t_0 and t_{24} (median and quartiles). *a*: GCF volume; *b*: IL-6 concentration; *c*: IL-6 total amount.

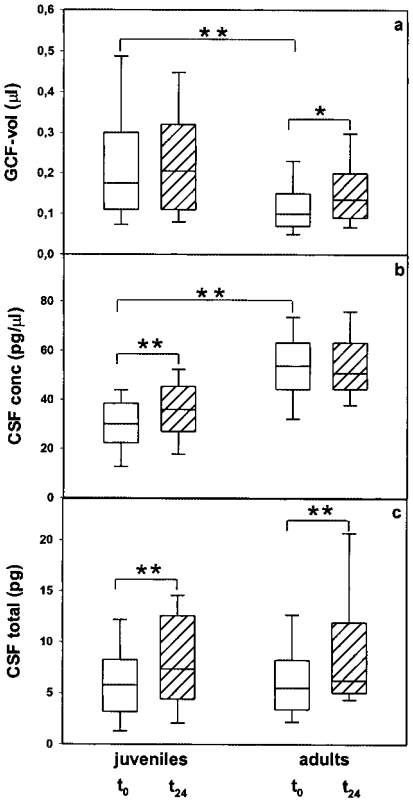

Fig. 3. Boxplots of GM-CSF levels in GCF samples in juveniles and adults from t_0 and t_{24} (median and quartiles). *a*: GCF volume; *b*: GM-CSF concentration; *c*: GM-CSF total amount.

of the mediators used in that study. The choice in later studies to report mediator concentrations has been based more on convenience than on scientific evidence (Grieve et al. 1994, Lowney et al. 1995, Uematsu et al. 1996a,b). Because of this controversy, we determined both the concentrations and total amounts of the three mediators.

Our results show that in juveniles the concentrations of all three mediators increased significantly from t_0 to t_{24}, but the volumes of these samples did not change. In adults, the GCF volumes increased from t_0 to $t_{24,}$ but the concentrations of IL-6 and GM-CSF remained the same. PGE_2, however, showed an increased concentration in adults. This is in agreement with the findings of Grieve et al. (1994). Increased levels of PGE_2 in GCF are also associated with increased severity and aggressiveness of periodontal disease (Offenbacher et al. 1984, Nakashima et al. 1994). The early increase of PGE_2 in tooth movement might be caused by an up-regulation of PGE_2 production directly after force application. This is in agreement with Davidovitch et al. (1988), who showed that the early phase of orthodontic tooth movement involves an acute inflammatory response. The increased production of other mediators may depend on the early up-regulation of PGE_2 system (Saito et al. 1990a, 1990b, Ngan et al. 1990).

In adults, the peak concentration for IL-6 and GM-CSF may be reached after more than 24 h; this is an interesting subject for further study. In juvenile patients, however, it has been shown before that IL-6 reaches a peak value already after 24 h of orthodontic force application (Uematsu et al. 1996b). The total amounts of the three mediators in juveniles and adults did not show any difference at t_0 and t_{24}. In both groups, the levels significantly increased.

These results indicate that mediator concentration is a more sensitive way to detect different responses in juveniles and adults. From a theoretical point of view as well, this seems reasonable, as the concentration of a mediator is more likely to trigger a response than the total amount. Therefore, in orthodontic tooth movement, bone remodelling is probably switched on by the concentration of certain mediators early on in the periodontium.

IL-6 and GM-CSF are both important inflammatory mediators. In juveniles their synthesis is up-regulated within 24 h of orthodontic force application, while in adults it probably takes more time. This suggests that in juveniles the inflammatory system is always in a more activated state and can therefore react faster to local changes. This is consistent with the clinical experience that juveniles have a stronger response to force activation during the initial stage of treatment (Bond 1972, Northway et al. 1984, Melsen 1991). On the other hand, concentrations of IL-6, GM-CSF and PGE_2 were significantly higher in adults than in juveniles at t_0, without any clinical signs of inflammation. This might indicate that higher levels of these mediators have to be generated to trigger inflammatory reactions in adults.

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. During this response, leucocytes as well as fibroblasts produce several inflammatory mediators, which are secreted into GCF. GCF analysis has been proven to be an effective method to study PDL and alveolar bone remodelling (Insoft et al. 1996, Tzannetou & Lamster 1998).

In conclusion, the analysis of mediator levels in GCF of periodontitis as well as of orthodontic patients is a valuable, non-invasive tool to study the condition of the periodontium. These mediator levels in GCF could possibly be used as reflecting parameters of efficiency of tooth movement in future.

Zusammenfassung

Cytokinlevel in der krevikulären Flüssigkeit sind weniger veränderbar bei Erwachsenen als bei Jugendlichen infolge orthodontischer Kräfte

Ziele: Knochenremodellation während orthodontischer Zahnbewegung steht in Beziehung zur Expression von Mediatoren in der gingivalen Sulkusflüssigkeit (GCF). Es gibt keine Informationen bezüglich des Effektes von Alter auf die Level dieser Mediatoren in der GCF. Der Zweck dieser Studie war die Quantifizierung von drei Mediatoren (Prostaglandin E_2, Interleukin-6 und Granulozyten-Makrophagen-Kolonien-stimulierender Faktor) in der GCF während orthodontischer Zahnbewegung bei Jugendlichen und Erwachsenen.

Material und Methoden: Insgesamt 43 jugendliche Patienten (mittleres Alter 11 ± 0.7 Jahre) und 41 erwachsene Patienten (mittleres Alter 24 ± 1.6 Jahre) nahmen an der Studie teil. Einer der lateralen Schneidezähne jedes Patienten wurde nach labial gekippt, der andere diente als Kontrolle. GCF Proben wurden vor der Aktivation (t_0) und 24 h später (t_{24}) gewonnen. Die Mediatorenlevel wurden mit Radioimmunoassay (RIA) bestimmt.

Ergebnisse: Die PGE_2 Konzentrationen waren signifikant zum Zeitpunkt t_{24} bei Jugendlichen und Erwachsenen erhöht, während die Konzentrationen von IL-6 und GM-CSF nur bei Jugendlichen signifikant erhöht waren. Die totale Menge der drei Mediatoren in der GCF nahmen signifikant zum Zeitpunkt t_{24} in beiden Gruppen zu.

Schlussfolgerungen: In der frühen Zahnbewegung sind die Mediatorenlevel bei Jugendlichen mehr veränderbar als bei Erwachsenen. Dies stimmt überein mit der Beobachtung, dass die initiale Zahnbewegung bei Jugendlichen schneller als bei Erwachsenen ist und ohne Verspätung beginnt.

Résumé

Les niveaux de cytokine dans le fluide gingival répondent moins aux forces orthodontiques chez l'adulte que chez les jeunes.

Objectifs: Le remodelage osseux lors des mouvements dentaires orthodontiques est en relation avec l'expression des médiateurs dans le fluide gingival (GCF). Aucune information n'est disponible sur les effets de l'âge sur les niveaux de ces médiateurs dans le GCF. Le but de cette étude était de quantifier 3 médiateurs (la prostaglandine E-2, L'interleukine-6 et le facteur de stimulation des colonies de macrophage et de granulocyte) dans le GCF pendant un mouvement orthodontique chez des jeunes et chez des adultes.

Matériels et méthodes: 43 jeunes patients (en moyenne âgés de 11 ± 0.7 ans) et 41 patients adultes (en moyenne âgés de 24 ± 1.6 ans) prirent part à cette étude. Une des incisives latérale de chaque patient fut versée vestibulairement, l'autre servant de contrôle. Des échantillons de GCF furent prélevés avant l'activation de la force (t0) et 24 heures plus tard (t24). Le niveau de cytokine fut déterminé par radioimmunoassay.

Résultats: Les concentrations de PgE-2 étaient significativement élevées à t24 chez les jeunes et les adultes, alors que les concentrations de IL-6 et de GM-CSF étaient significativement augmentées seulement chez les jeunes. La quantité totale des 3 médiateurs dans le GCF augmentait significativement à t24 dans chaque groupe.

Conclusions: Rapidement après l'application des mouvements dentaires, les niveaux de médiateurs chez les jeunes répondent plus que chez l'adulte, ce qui est en accord avec les résultats qui montrent que le mouvement initial dentaire chez les jeunes est plus rapide que chez les adultes et débute sans délai.

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