Original Article

Elevated levels of Interleukin (IL)-1β, IL-6, tumor necrosis factor-α, epidermal growth factor, and β2-microglobulin levels in gingival crevicular fluid during human Orthodontic tooth movement (OTM)

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Abstract

Aim: The aim of this study was to identify and quantify the various cytokines in human gingival crevicular fluid (GCF), and to investigate the changes in their levels during orthodontic tooth movement (OTM). **Materials and Methods:** A statistically significant no. of subjects (n = 10 and mean age = 15.6 years) were included in the study. A maxillary cuspid of each subject having one treatment for distal orthodontic tooth movement served as the experimental tooth, whereas the contralateral cuspids were used as controls. Gingival crevicular fluid (GCF) around the experimental and the two control teeth was collected from each subject immediately before activation, and at 1, 24, and 168 hours after the initiation of tooth movement. **Result:** ELISAs were used to determine cytokine levels. The concentrations of interleukin (IL)-1lβ, IL-6, tumor necrosis factor-α, epidermal growth factor, and β2-microglobulin were significantly higher in the experimental group than in the controls at 24 hr after the experiment was initiated. All the cytokines remained at baseline levels throughout the experiment for the control groups. **Conclusion:** Since all cytokines in GCF play an important role in the bone remodelling processes *in vivo*, the present results indicate that the changes in cytokines in GCF are associated with OTM.

Keywords: EGF, IL1β, IL-6, TNF-α, β2-MG orthodontic tooth movement

Introduction

The early phase of orthodontic tooth movement (OTM) involves an acute inflammatory response both at biochemical and structural level, characterized by periodontal vasodilatation and the migration of leukocytes out of periodontal ligament (PDL) capillaries.[1] Orthodontic tooth movement (OTM) is regarded as an epiphenomenon of the gene expression of the periodontal ligament (PDL) and surrounding cells resulting from a series of orchestrated cellular and molecular events in alveolar bone and periodontal tissue initiated by the application of orthodontic force.[2] The mechanism of bone resorption may also be related to release of inflammatory mediators, such as prostaglandin E (PGE) and interleukin (IL)-1, which interact with bone cells. Cytokines

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secreted by leukocytes may interact directly with osteoblasts or indirectly via neighboring cells, such as monocytes/ macrophages, lymphocytes, and fibroblasts, through their production of cytokine, or a variety of growth factors. A chemical cascade that mediates the transmission of signals from extracellular matrix leading to genetic modulation is interceded by the release of mediators in paracrine environment. These signals are responsible for a change in the cytoskeletal structure, leading to alteration of nuclear protein matrix and eventually gene activation or suppression.[3,4] These events initiate the process of bone remodelling, leading to effective tooth movement. The biochemical mediators released sequentially at multiple stages during orchestration of tooth movement can be detected in gingival crevicular fluid (GCF). GCF is a unique biological exudate that has been found as a convenient medium to study these mediators with reasonable sensitivity. GCF can be collected noninvasively[5] with specifically designed filter paper or a micropipette (1 to 10 μl) or through magnetic beads placed in gingival crevice. Once collected, GCF may be cryopreserved or directly sent for chemical analysis. GCF can also be collected repeatedly at various stages of orthodontic treatment and therefore provides useful insight to biological events over the entire duration of observation. Clinically demonstrable success of OTM is associated with expression of numerous regulatory molecules, of which cytokines have been most widely documented. Cytokines are low-molecular weight proteins (mw <25 kDa) released in autocrine or paracrine environments in response to local signals like application of stress[6] and are involved in normal physiological bone turnover and remodelling.[7-9] Cytokine biology as a retort to forces applied for OTM is difficult to comprehend due to sheer number and complexity of these factors exhibiting redundancy as well as pleiotropy.[10] Although cytokines have been extensively evaluated in GCF as quantitative biochemical indicators of inflammatory periodontal status,[11] there has been an increasing interest on understanding their contributions as mediators of OTM owing to their role in bone and tissue remodelling. Among these cytokines, interleukins (ILs) (IL-1α, IL-1β, IL-1RA, IL-8, IL-2, IL-6, and IL-15), tumor necrosis factors (TNFs), interferons (IFNs), growth factors (GFs), and colony stimulating factors (CSFs) have been extensively studied in relation to OTM.

Aim

This study was designed to investigate the levels of IL1β, IL-6, TNF-α, EGF, and P2-MG in GCF at 0, 24, and 168 hours after the start of orthodontic treatment, by use of highly sensitive enzyme immunoassays (EIA). The present study demonstrates that cytokines in GCF are rapidly elevated at 24 hours after the orthodontic force is initiated and suggests that these alterations of cytokines are associated with bone remodelling processes occurring during orthodontic tooth movement *in vivo*.

Materials and Methods

Study population

Ten orthodontic patients (seven females and three males; mean age, 15.6 + 0.9 years) were selected to participate in this study according to the following criteria: (1) good systemic health; (2) not on antibiotic therapy within the past 6 months; (3) no use of anti-inflammatory drugs in the month preceding the study; (4) healthy periodontium, with generalized probing depth of 2 mm and no radiographic evidence of periodontal bone loss; and (5) requirements of first-premolar extraction and canine distal tooth movement as a part of orthodontic treatment. Informed consent was obtained from each patient. The human subject's protocol was reviewed and approved by Institutional Ethical Review Board.

Experimental design

For each subject, a maxillary cuspid undergoing distal orthodontic tooth movement was used as an experimental tooth, and the contralateral cuspids served as control tooth. Orthodontic brackets were placed on the canines. Experimental canines were moved in the distal direction through an archwire by use of an elastic chain exerting an initial force of 250 g. The amount of tooth movement for each tooth was measured with digimaticcalipers. At the distal aspect of experimental and control teeth, GCF was collected for subsequent analysis and the following examinations of the periodontium were conducted: Probing depth, presence or absence of plaque, and bleeding on probing. The collection and examinations were conducted immediately before activation and at 1, 24, and 168 hours after the initiation of tooth movement.

GCF collection

The GCF sampling was performed by the method of Offenbacher *et al.*[12] GCF was collected from the experimental and control teeth. The tooth was gently washed with water, and the sites under study were isolated with cotton rolls (to minimize contamination from saliva) and gently dried with an air syringe. Paper strips (Periopaper, Harco, Tustin, CA, USA) were carefully inserted 1 mm into the gingival crevice and allowed to remain there for 30 seconds. After a one-minute interval, a second strip was placed at the same site. Care was taken to avoid mechanical injury. The volume of GCF in the periopaper was measured with a Periotron (Harco, Tustin, CA, USA). The paper strips from the individual sites were stored at -30°C until further processing could be carried out.

IL1β, IL-6, TNF-α, EGF, and β2-MG in GCF

To obtain the sample completely from periopaper, we eluted the GCF from the paper strips by centrifugal filtration with aliquots of buffer (50 mm phosphate buffer, pH 7.2, containing protease inhibitors, 0.1 mm phenylmethylsulfonyl fluoride, 50 μg/mL each of leupeptin, pepstatin, and antipain). In brief, 100 μL of the above buffer was applied to each strip and the tube centrifuged at 15,000 g for 5 minutes. A further 100 μL was then applied and the centrifugation repeated. The GCF from the two strips was pooled to give a total volume of 200 μL and then stored at -30°C for later assay. The pilot study for estimation of recovery showed that from 83-91% of the initial amount of bovine serum albumin (BSA) applied could be recovered by our method. Protein concentration of the extract was estimated by the method of Bradford, with BSA as a standard.[13]

The contents of IL-11 and IL-6 in the samples were measured by use of commercially available two-site sandwich ELISA kits. EGF was measured by a two-site ELISA. TNF-α and β2-MG were determined by a sandwich EIA that consisted of solid-phase (polystyrene bead) immobilized antibodies and antibodies labelled with 3-Dgalactosidase, as described previously.[14-16] All samples and standards were assayed in duplicate.

Results

The amount of tooth movement was 1.2 ± 0.1 mm/168 hours on the average, whereas no movement was detected on the control site. GCF has been co-related with the inflammatory state.[17] As for the volume of GCF samples during orthodontic tooth movement, the mean GCF from the experimental tooth at 24 hours (0.51 ± 0.05μL/2 periopapers) was a little higher than from the experimental site at 24 hours (0.37 ± 0.05 μL/2 periopapers); however, there was no statistically significant difference due to the great variation. In addition, the volume of GCF from around the experimental tooth was similar to that of GCF samples ((0.41 ± 0.05μL/2 periopapers) from healthy subjects.

IL1β, IL-6, TNF-α, EGF, and β2-MG contents in GCF

IL1β: The meanIL1β for experimental teeth at 24 hours was considerably higher compared to baseline (0.89 ± 0.12 pg/μg vs 0.37 ± 0.11 pg/μg, $P < 0.05$*) and also in contralateral control teeth (0.89 ± 0.12 pg/μg vs 0.34 ± 0.11 pg/μg, $P < 0.05$*) [Table 1].

IL-6: The mean IL-6 value for experimental teeth at 24 hours was considerably higher compared to baseline (0.065 ± 0.005 pg/μg vs 0.030 ± 0.008 pg/μg, $p < 0.05$*) and also in contralateral control teeth (0.016 ± 0.006 pg/μg vs 0.034 ± 0.0091 pg/μg, $p = 0.00145$*) [Table 2].

TNF-α: The mean TNF-αfor experimental teeth at 24 hours was considerably higher compared to baseline (1.18 ± 0.05 pg/μg vs 0.43 ± 0.15 pg/μg, $p < 0.05$*) and also in contralateral control teeth (0.45 ± 0.12 pg/μg vs 0.34 ± 0.11 pg/μg, $p < 0.05$*) [Table 3].

EGF: The mean IL1β for experimental teeth at 24 hours was considerably higher compared to baseline (0.84 ± 0.12 pg/μg vs 0.18 ± 0.11 pg/μg, $p < 0.05$*) and also in contralateral

Table 1: Mean IL1β results

Time-interval	Experimental	Control	*P*	Significance
At baseline	0.37±0.11 pg/μg	0.34±0.11 pg/μg	*P*>0.05	(non-significant)
At 24 hr	0.89±0.12 pg/μg	0.89±0.12 pg/μg	*P*>0.05	(non-significant)
P (base vs 24)	*P*<0.05*	*P*<0.05*		*(significant)
At 168 hr	0.45±0.13 pg/μg	0.43±0.11 pg/μg		
P (base vs168)	*P*>0.05 (non-significant)	*P*>0.05 (non-significant)		

Table 2: Mean IL 6 results

Time-interval	Experimental	Control	*P*	Significance
At baseline	0.030±0.008 pg/μg	0.034±0.0091 pg/μg	*P*>0.05	(non-significant)
At 24 hr	0.065±0.005 pg/μg	0.016±0.006 pg/μg	*P*<0.05	(significant)
At 168 hr	0.044±0.007 pg/μg	0.036±0.0091 pg/μg	*P*>0.05	(non-significant)
P (base vs 24)	*P*=0.044*	*P*=0.00145	*P*<0.05	*(significant)
P (base vs 168)	*P*>0.05 (non-significant)	*P*>0.05 (non-significant)		

Table 3: Mean TNF-α results

Time-interval	Experimental	Control	*P*	Significance
At baseline	0.43±0.15 pg/μg	0.34±0.11 pg/μg	*P*>0.05	(non-significant)
At 24 hr	1.18±0.05 pg/μg	0.45±0.12 pg/μg	*P*<0.05	(significant)
At 168 hr	0.45±0.05 pg/μg	0.36±0.11 pg/μg		
P (base vs 24); (base vs 168)	*P*<0.05*(significant); *P*>0.05 (non-significant)	*P*>0.05(non-significant)		

Table 4: Mean EGF results

Time-interval	Experimental	Control	*P*	Significance
At baseline	0.18±0.11 pg/μg	0.17±0.12 pg/μg	*P*>0.05	(non-significant)
At 24 hr	0.84±0.12 pg/μg	0.31±0.16 pg/μg	*P*<0.05*	(significant)
At 168th hr	0.21±0.14 pg/μg	0.19±0.13 pg/μg	*P*>0.05	(non-significant)
P (base vs 24); (base vs 168)	*P*<0.05* *(significant); *P*>0.05(non-sig.)	*P*<0.05* *(significant); *P*>0.05(non-sig.)		

Table 5: Mean β2-MG results

Time-interval	Experimental	Control	*P*	Significance
At baseline	0.37±0.11 pg/μg	0.34±0.11 pg/μg	*P*>0.05	(non-significant)
At 24 hr	8.9±4.1 pg/μg	0.49±3.4 pg/μg	*P*<0.05*	(significant)
At 168 hr	16.4±4.8 pg/μg	0.63±0.11 pg/μg	*P*<0.05*	(significant)
P both at base vs 24 and 168	*P*<0.05*	*P*<0.05*		*(significant)

control teeth (0.31 ± 0.12 pg/μg vs 0.17 ± 0.11 pg/μg, $p < 0.05$*) [Table 4].

β2-MG: The mean β2-MG gradually increased to its maximum level at 168 hours (16.4 ± 4.8 pg/μg), and a statistically significant difference was found between experimental and control site at 24 hours (8.9 ± 4.1 pg/μg vs. 0.49 ± 3.4 pg/μg) [Table 5].

Discussion

The altering levels, rise, and fall of the mediators in GCF are suggestive of underlying intricate biological remodelling processes in bone and periodontal tissues that eventually leads to OTM. The forces employed for OTM led to an initial increase in levels of inflammatory mediators as well as associated receptors namely IL-1β, IL-6 and TNF-α as early as 1 minute[16] or 1 hour[18] and attained peak in 24 hours.[17-31] These mediators slowly decrease to baseline in subsequent observation points at 48 hours, 168 hours, 14 days, and 21 days.[18,31] The present study has reported the cytokine levels (pg) per μg of total protein in the GCF. This is an important point because: (1) fluidcontents in GCF varyvastlybetween samples; (2) all values of cytokine (pg) per μg of protein in GCF at the stressed sites showed a rapid and transient increase, as compared with those for both control sites at 24 hours – or experimental site at baseline (0 h r) further confirming cytokine induction during tooth mobilization.

It has been speculated that the cytokines are produced by the cells of PDL and alveolar bone, such as fibroblasts, macrophages, osteoblasts and osteoclasts. Mechanically deformed osteoblasts and PDL cells display a wide range of molecular alterations, some of which are capable of causing bone resorption.[20] They include IL-1β, TNFα, and IL-6 which have been implicated in bone resorption processes.[18,23,26] Inflammation also involves the elevation of β2-MG which has close association with major histocompatibility complex-I and play a major role in inflammation.

Conclusion

The present study thus concludes that all cytokines in GCF play an important role in the bone remodelling processes *in vivo*. The present results indicate that the changes in cytokines in GCF are associated with orthodontic tooth movement.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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