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# Relationship between substance P and interleukin-1β in gingival crevicular fluid during orthodontic tooth movement in adults

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SUMMARY Metabolism by peptidases plays an important role in modulating the levels of biologically-active neuropeptides, while that of substance P (SP), a component of gingival crevicular fluid (GCF), may potentiate the inflammatory process in orthodontic tooth movement. The aim of this study was two-fold: (1) to investigate GCF levels of SP and interleukin-1β (IL-1β) during human orthodontic tooth movement, and (2) to determine the correlation coefficients between SP and IL-1β levels in the GCF.
The subjects were 3 males, with a mean age of 21.3 ± 2.8 years old, and 6 females, with a mean age of 23.1 ± 2.4 years, undergoing orthodontic movement of a single tooth, with the contralateral tooth used as the control. GCF was sampled at the control and treatment (compression) sites before and 1, 4, 8, 24, 72, 120, and 168 hours after initiation of orthodontic treatment. Prevention of plaque-induced inflammation allowed assessment of the dynamics of mechanically stimulated SP and IL-1β levels in the GCF, which were determined using enzyme-linked immunosorbent assay (ELISA) kits.
GCF levels of SP and IL-1β for the treated teeth were significantly higher ($P < 0.001$) than for the corresponding control teeth from 8 to 72 hours, and peaked at 24 hours.
These results show that the amounts of SP and IL-1β in GCF increase with orthodontic tooth movement, and indicate that such increases may be involved in inflammation in response to mechanical stress.

## Introduction

The peripheral sensory nervous system contributes to the development of acute and chronic inflammatory processes through local release of neuropeptides. A number of different neuropeptides, including substance P (SP), are known to be present in the nerve fibres that supply the tooth pulp and periodontium in rats, cats, monkeys, and humans (Olgart and Gazelius, 1977; Wakisaka *et al*., 1985; Silverman and Kruger 1987; Byers *et al*., 1987; Luthman *et al*., 1988; Casasco *et al*., 1990). Further, Norevall *et al*. (1995) reported that the expression of SP was increased after orthodontic tooth movement in rat periodontal ligament (PDL) specimens. These observations suggest that SP is involved with remodelling of the PDL and alveolar bone during orthodontic tooth movement.

Biologically active substances, such as cytokines and enzymes, are expressed by cells within the periodontium in response to mechanical stress from orthodontic appliances (Davidovitch *et al*., 1988; Saito *et al*., 1991). The overall objective of many investigations has been to better understand the mechanisms for converting physical stress to the cellular responses that occur during tooth movement. In order to monitor the expression of biologically active substances in humans in a non-invasive manner, changes in the composition of gingival crevicular fluid (GCF) during orthodontic tooth movement have also been studied (Griffiths *et al*., 1998; Iwasaki *et al*., 2001). These substances are involved in bone remodelling and produced by PDL cells, and then diffusely excreted in sufficient quantities into the GCF.

Interleukin (IL)-1β is a key mediator involved in a variety of immune and acute-phase inflammatory response activities (Dinarello, 1989), as well as bone resorption (Gowen *et al*., 1985), and has been identified in the GCF during orthodontic tooth movement (Uemastsu *et al*., 1996). Further, SP has been reported to induce the secretion of IL-1β from monocytes (Lotz *et al*., 1988). However, little information is available concerning the relationship between SP and IL-1β in the GCF during orthodontic tooth movement in humans.

Therefore, in the present study, the aims were to investigate the levels of SP and IL-1β in GCF samples before and, 1, 4, 8, 24, 72, 120, and 168 hours after the initiation of orthodontic treatment, using commercially enzyme-linked immunosorbent assay (ELISA) kits.

## Subjects and methods

### *Subjects*

Informed consent from the subjects was obtained after an explanation of the study protocol, which was reviewed by the Board of Nihon University School of Dentistry at Matsudo.

Nine adult orthodontic patients (3 males, mean age 21.3 ± 2.8 years; 6 females, mean age of 23.1 ± 2.4 years) were enrolled in the study, after meeting the following criteria: (1) good general health; (2) lack of antibiotic therapy during the previous 6 months; (3) absence of anti-inflammatory drug administration in the month preceding the study; (4) healthy periodontal tissues with generalized probing depths ≤3 mm and no radiographic evidence of periodontal bone loss; and (5) first premolar extraction and canine distal tooth movement as part of the orthodontic treatment plan.

*Experimental design*

The experiment was performed using the method of Sugiyama *et al*. (2003). In each subject, the upper first premolars were extracted before placing the brackets and wire. The canine undergoing distal movement was used as the experimental tooth and the contralateral canine served as the control. Orthodontic brackets were placed on both the treatment and control teeth using an edgewise technique, in which 0.018 × 0.025-inch slot bands and brackets (Tomy International Inc., Tokyo, Japan) were used. The canines were retracted with elastomeric chains (Tomy International Inc.) on a 0.018-inch round wire (Tomy International Inc.).

The experimental canines were moved in a distal direction using an archwire with an elastic chain that exerted an initial force of 250*g*. The contralateral canines were not subjected to orthodontic force. The amount of movement required for each tooth was measured with digimatic callipers. After impression taking and production of working models, measurements were performed using an electronic digital calliper (Max-Cal; Japan Micrometer MFG Co. Ltd, Tokyo, Japan) that had an accuracy of 0.01 mm. Errors associated with measuring tooth movement were negated by measuring 10 times. At the distal aspects of both the experimental and control teeth, GCF was collected for subsequent analysis, and the following periodontal examinations were conducted: probing depth, presence or absence of plaque, and bleeding on probing. Thereafter, GCF samples were collected before and, 1, 4, 8, 24, 72, 120, and 168 hours after initiation of tooth movement.

*GCF collection*

Prior to GCF collection, gingival inflammation was assessed by recording the colour of the gingivae, and plaque according to the index proposed by Silness and Löe (1964). Since plaque is known to contaminate GCF samples (Griffiths *et al*., 1992), light deposits were removed with a periodontal probe, while a sickle scaler was utilized for heavier deposits.

Following GCF collection, probing depth and attachment level were recorded, to ensure that periodontal breakdown had not occurred during the study, and bleeding following probing was also noted as a further assessment of inflammation.

GCF sampling was performed using the method of Offenbacher *et al*. (1986), and collected from both experimental and control teeth at the same time. The teeth were gently washed with water, and the sites under study were isolated with cotton rolls (to minimize saliva contamination) and gently dried with an air syringe. Paper strips (Periopaper, Harco, Tustin, California, USA) were carefully inserted 1 mm into the gingival crevice and allowed to remain there for 1 minute, after which a second strip was placed at the same site. Care was taken to avoid mechanical injury. The volume of GCF on each paper strip was measured using a Periotron 8000 (Harco) that was calibrated with human serum. GCF collection for a fixed amount of time allowed for standardization of site and subject differences. Paper strips from individual sites were stored at –30ºC until further processing.

Prior to evaluation, the paper strips were placed individually in 100 µl of Tris buffer (12 mM Tris, containing 0.1 M NaCl and 0.05 per cent Tween 20) and then subjected to a ×3 vortex over 30 minutes. The strips were then removed and the eluate was centrifuged for 5 minutes at ×3000*g*, after which the supernatants were separated and frozen at –30ºC for later use. Protein concentrations in the extracts were estimated by the method of Bradford (1976), with bovine serum albumin used as the standard.

*Enzyme immunoassay*

SP and IL-1β levels were measured in duplicate using a commercial ELISA kit (Quantikine, R&D Systems Inc., Minneapolis, Minnesota, USA), with the results expressed in pg/µg of total protein in the GCF.

*Statistical methods*

The values were calculated as the mean ± standard deviation (SD) and Mann-Whitney *U*-tests were used to compare the means of the groups. Relationships between two variables were assessed by Spearman's signed rank test. Statistical analysis was performed using the Statistica (V5.5, StatSoft Japan Inc., Tokyo, Japan) computer program.

## Results

*Clinical parameters*

The average amount of tooth movement was 1.5 ± 0.4 mm over 168 hours (7 days). There was no movement detected for either of the control teeth in any of the subjects. GCF volume has been shown to be correlated with inflammation (Cimasoni, 1983); however, there was no significant difference in the mean volume of GCF at any time point between the experimental (0.40 ± 0.06 µl) and control teeth (0.39 ± 0.07 µl) when collected on paper strips. In addition, the volume of GCF from around each experimental tooth was similar to that of the GCF samples from the untreated

control teeth (0.40 ± 0.05 μl). In all patients, plaque accumulation was minimal throughout the entire study and gingival health was excellent, with no gingival bleeding. Furthermore, probing depths remained less than 2 mm at all times throughout the experimental period.

*Levels of SP and IL-1β in GCF*

Following orthodontic activation, significant differences were found between the control and treated teeth, as the mean SP and IL-1β values for the treated teeth were significantly higher after 8, 24, and 72 hours. However, there were no significant differences between the experimental site and the baseline measurements at 168 hours ( Figures 1 and 2).

In contrast to the changes in SP and IL-1β levels, there were no significant differences in total protein level at any of the experimental time periods between the different experimental sites, or between the experimental and control sites of individual patients (data not shown). Therefore, the protein pattern was clearly different from that of SP and IL-1β during orthodontic tooth movement.

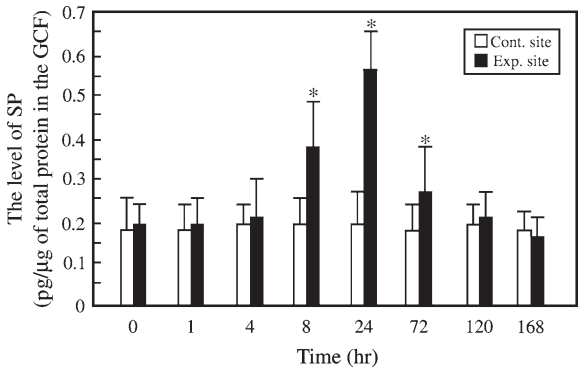


**Figure 1** Changes in substance P (SP) concentration in gingival crevicular fluid (GCF) samples during orthodontic tooth movement. *$P < 0.001$.

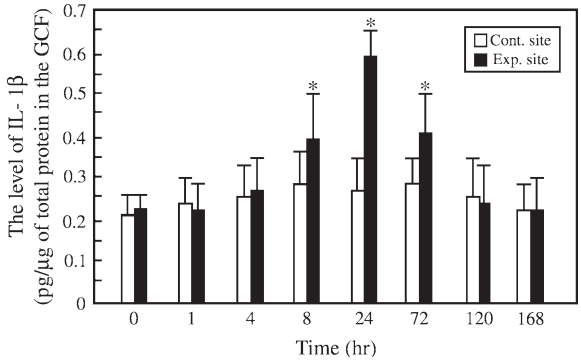


**Figure 2** Changes in interleukin-1β (IL-1β) concentration in gingival crevicular fluid (GCF) samples during orthodontic tooth movement. *$P < 0.001$.

*Spearman's correlation coefficients*

Table 1 shows Spearman's correlation coefficients between SP and IL-1β in the GCF samples. There was a significant correlation between the two at 8, 24, and 72 hours.

## Discussion

The results of the biochemical analysis of GCF, an osmotically mediated inflammatory exudate found in the gingival sulcus, showed the present method to be effective for monitoring and for early detection of periodontal disease (Lamster *et al*., 1988a, b). As an exudate, the amount of fluid in any crevicular location tends to increase with inflammation and capillary permeability (Brill and Bjorn, 1959). Furthermore, orthodontic forces induce the movement of PDL fluids and, with them, any cellular biochemical product produced from prior mechanical perturbation. The direction of flow of the PDL fluid may occur as follows: from an area of compression to an area of tension, in both apical and coronal directions, toward the gingival sulcus and/or into the alveolar marrow spaces. Therefore, compression of the PDL may cause cellular biochemical by-products to appear in the sulcus. In addition, the effect of orthodontic force on the PDL is rapid, as the migration of carbon particles out of the capillaries in the compressed PDL has been shown to occur within minutes of the application of force to guinea pig incisors (Storey, 1973).

As a result of the application of mechanical force, PDL cells may produce sufficient amounts of modulators during bone remodelling and extracellular matrix metabolism, and then be diffusely secreted into the GCF. It has been reported that modulators, including cathepsin B and L, and tissue type plasminogen activity and plasminogen activity inhibitor-2, which are secreted into the GCF, are elevated during orthodontic tooth movement (Nozoe *et al*., 2002; Sugiyama *et al.*, 2003; Hoshino-Itoh *et al.*, 2005). Therefore, it is speculated that the elevation of inflammatory indicators in GCF flow reflects these comprehensive biological responses that are induced by mechanical stress.

With regard to sample age in the present study, this may be regarded as high (3 males, mean age 21.3 ± 2.8 years; 6 females, mean age of 23.1 ± 2.4 years). Ren *et al.* (2002)

**Table 1** Spearman's ranked correlation coefficient between substance P (SP) and interleukin-1β (IL-1β) in gingival crevicular fluid (GCF).

| SP versus IL-1β | Correlation | |
|---|---|---|
| | r | *P* |
| 8 hours | 0.889 | 0.001 |
| 24 hours | 0.905 | 0.001 |
| 72 hours | 0.865 | 0.001 |

reported that inflammatory mediator levels ($PGE_2$, IL-6, and granulocyte-macrophage colony-stimulating factor) in juveniles (mean age 11 ± 0.7 years) are more responsive than levels in adults (mean age 24 ± 1.6 years); this approach may be suitable for studying how inflammatory mediator levels in the GCF flow of juveniles respond to orthodontic tooth movement. However, whilst the request for orthodontic treatment in adults is increasing, little information on inflammatory mediators in GCF during orthodontic tooth movement is available. Therefore, the levels of SP and IL-1β in GCF during orthodontic tooth movement in adults were investigated.

The *in vivo* findings of this study showed a statistically significant elevation of SP in GCF samples from areas adjacent to teeth undergoing orthodontic tooth movement, as the mean total levels were increased significantly at 8, 24, and 72 hours after initiating orthodontic force, by approximately 2.1-, 2.9-, and 1.6-fold, respectively, compared with the control teeth. However, after 120 and 168 hours, SP levels were nearly the same as those measured at baseline. The present application design did not provide for continuous forces at 120 and 168 hours, which may explain why SP was not elevated at those time points ( Figure 1). The GCF flow of SP during orthodontic tooth movement has not previously been reported. Lundy *et al.* (2000) found that SP in GCF in subjects with periodontitis was higher (42.4 pg) than in healthy subjects (2.0 pg). Periodontitis is a disease that results in the destruction of the structures that support the teeth, namely alveolar bone and connective tissue attachment to teeth. Orthodontic tooth movement is not a disease and is not associated with bone attachment loss. Therefore the GCF flow of SP during orthodontic tooth movement may be lower than that found in subjects with periodontitis. Additional studies are necessary to clarify the relationship between the GCF flow of SP during orthodontic tooth movement in adults and periodontitis.

In an experimental study (Nicolay *et al*., 1990), SP appeared to increase markedly after application of orthodontic force in cats, which occurred rapidly (after 3 hours) in the dental pulp, though later in the PDL (after 24 hours). In addition, a recent detailed time period study reported that GCF matrix metalloproteinase (MMP)-8 levels for teeth undergoing orthodontic treatment were significantly higher at 4 to 8 hours after force application compared with that prior to initiation (Apajalahti *et al*., 2003). The results of the present investigation may support the findings of those previous studies, as the mean total levels of IL-1β were increased significantly at 8, 24, and 72 hours after initiating orthodontic force, by approximately 1.5-, 2.2-, and 1.7-fold, respectively, compared with the controls ( Figure 2). In addition, previous studies have demonstrated that the levels of IL-1β are increased compared with those at the control sites after 24 hours (Grieve *et al*., 1994; Uemastsu *et al*., 1996). The present findings at 24 hours, which clearly showed that the levels of IL-1β in the GCF were increased during orthodontic tooth movement are in agreement with the results of detailed time period studies. With regard to an increase in the GCF flow of IL-1β, Uematsu *et al.* (1996) reported that at 24 hours, mean GCF IL-1β levels were significantly elevated at treatment teeth (0.88 ± 0.11 pg/μg) compared with the control teeth (0.38 ± 0.07 pg/μg). These results (treatment teeth versus control teeth; 0.58 ± 0.08 pg/μg versus 0.26 ± 0.09 pg/μg) in the present study agree with their findings.

There was also a significant correlation between SP and IL-1β levels in the GCF at 8, 24, and 72 hours ( Table 1). It is becoming increasingly evident that periodontitis as well as other orofacial inflammatory disorders may be modulated by imbalances in certain neuropeptides. Luthman *et al*. (1989) suggested that SP plays an important role in the pathogenesis of periodontitis, while SP levels were significantly elevated in the GCF of disease-affected teeth in periodontitis-affected subjects, as compared with healthy sites (Linden *et al*., 1997). Furthermore, Hanioka *et al.* (2000) reported that SP showed significant correlation with IL-1β of the host response in GCF from periodontitis. Recent studies have also shown that SP induces the production of tumour necrosis factor-α, IL-1β, and IL-6 by T-lymphocytes, macrophages, neutrophils, and dental pulp cells (Delgado *et al*., 2003; Yamaguchi *et al*., 2004). These findings suggest that SP participates in the complex network of mediators that regulate inflammation. Therefore, increased levels of IL-1β in GCF may be initiated by SP in response to orthodontic force.

Neurokinin A, as well as SP, is a member of the tachykinin (tachy-swift) neuropeptide family, which evoke rapid responses upon release, and calcitonin gene-related peptide, vasoactive intestinal polypeptide, and Neuropeptide Y innervate human periodontal tissues (Luthman *et al*., 1988). These have been detected in GCF samples (Linden *et al*., 1997, 2002; Lundy *et al*., 1999) and it has been suggested that they may play an important role in maintaining periodontal health. Further studies regarding these neuropeptides in GCF during orthodontic tooth movement are needed.

With regard to the relationship between neuropeptides and pain during orthodontic treatment, Furstman and Bernick (1972) suggested that periodontal pain is caused by the processes of pressure, ischaemia, inflammation, and oedema. Further, Burstone (1964) identified both immediate and delayed pain responses, with the former response related to the initial compression of the PDL immediately after placement of the archwire and the latter, which began a few hours later, and which was termed hyperalgesia of the PDL, was caused by an increased sensitivity of nerve fibres to noxious stimuli such as prostaglandins, histamines, and SP, a neuropeptide released from nociceptors in the region of tissue damage that increases the firing rate of neurons that relay nociceptive information. Erdinc and Dincer (2004) reported that perception of pain during orthodontic

treatment with a fixed appliance reached a peak at 24 hours and decreased by day 3, which suggests that the perception of pain may be linked to the release of SP following orthodontic tooth movement.

## Conclusions

In the present study, it was found that the levels of SP and IL-1β in GCF samples were increased by orthodontic tooth movement in adults, and were considered to be involved in periodontal inflammation as a response to mechanical stress.

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