Painful Tooth Stimulation Elevates Matrix Metalloproteinase-8 Levels Locally in Human Gingival Crevicular Fluid

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J DENT RES 2005 84: 335

DOI: 10.1177/154405910508400408

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J Dent Res 84(4):335-339, 2005

ABSTRACT

Recent studies have demonstrated that pulpal pain can induce neurogenic inflammatory reactions in gingiva and the expression of pro-inflammatory neuropeptides in gingival crevicular fluid (GCF). Neuropeptides co-ordinate the activity of immune-effector cells and may influence the secretion of matrix metalloproteinase (MMP)-8, the major tissue-destructive protease in GCF. With this background, we studied whether experimental pulpal pain can trigger changes in GCF MMP-8 levels. The molecular forms of MMP-8 in the GCF of stimulated and non-stimulated teeth were analyzed by Western immunoblot, and MMP-8 levels by quantitative immunofluorometric assay. Painful stimulation of the upper incisor provoked significant elevations in GCF MMP-8 levels of the stimulated tooth. Western immunoblot revealed elevations in both neutrophil- and mesenchymal-type MMP-8 isoforms. At the same time, the GCF MMP-8 levels of the non-stimulated teeth were not changed. Analysis of these data indicated that pulpal pain can induce local elevations in MMP-8 levels in GCF.

KEY WORDS: pulpal pain, matrix metalloproteinase-8, gingival crevicular fluid, neurogenic inflammation.

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INTRODUCTION

Oral bacteria and their toxins, enzymes, and metabolites are largely responsible for the initiation of periodontal inflammation (Listgarten, 1987). Research has shown that chemical stimulation of gingival nociceptive afferents (Fazekas *et al.*, 1990; Kempainen *et al.*, 2003) and painful tooth stimulation (Kempainen *et al.*, 2001, 2003) can induce similar inflammatory reactions in gingivo-mucosal tissues. This process can release inflammatory mediators from the peripheral nerve terminals, and is thus called 'axon reflex-mediated neurogenic inflammation' (Holzer, 1988). Interestingly, such neurogenic mechanisms may be responsible not only for pulpal-pain-induced blood flow elevations in gingiva (Kempainen *et al.*, 2003), but also for neuropeptide level elevations in gingival crevicular fluid (GCF) (Awawdeh *et al.*, 2002a).

At local sites of various tissues, sensory nerves are closely apposed to immune-effector cells, including neutrophils, monocytes/macrophages, and fibroblasts (Stead *et al.*, 1989; McGillis and Fernandez, 1999). Clinically, the pro-inflammatory neuropeptides released by sensory nerves co-ordinate the activities of the immune-effector cells (McGillis and Fernandez, 1999), and may play an important role in the pathogenesis of periodontitis (Luthman *et al.*, 1989). Painful teeth with pulpitis have been shown to be associated with elevated levels in GCF of pro-inflammatory peptides, such as substance P (SP) and neurokinin A (NKA) (Awawdeh *et al.*, 2002a). Matrix metalloproteinase (MMP)-8, released by neutrophils, monocytes/macrophages, and fibroblasts, is the major pivotal tissue-destructive protease, especially in the periodontitis-affected gingiva and gingival crevicular fluid (GCF) (Sorsa *et al.*, 1988, 2004; Kiili *et al.*, 2002; Mäntylä *et al.*, 2003). Recently, research has shown that MMP-8, in addition to its surrogate catalytic action (Uitto *et al.*, 2003; Sorsa *et al.*, 2004), can also exert anti-inflammatory or defensive characteristics (Owen *et al.*, 2004).

With this background, we studied whether painful tooth stimulation can induce changes in GCF MMP-8 levels in humans. To evaluate the spatial spread of pulpal-pain-evoked effects, we analyzed the MMP-8 levels in GCF collected from the crevices of the stimulated upper incisors and of non-stimulated upper and lower incisors.

MATERIALS & METHODS

Subjects

Eight trained volunteers (five males, three females), ranging in age from 24 to 45 yrs, were tested in the experiments in our study. The subjects were healthy graduate students or researchers, who were in excellent oral and general health and were free of clinical signs of infection in their oral tissues. Informed consent was obtained from each subject prior to the experiments, according to the ethical guidelines of the Helsinki Declaration of 1975. The Ethics Committee of the Medical Faculty of the University of Helsinki approved the study protocol.

Received March 22, 2004; Last revision December 9, 2004;
Accepted January 18, 2005

Stimulation Techniques

The dental stimulation was generated with a constant-current tooth stimulator as previously described (Kempainen *et al.*, 1985). During the experimental sessions, the permanent upper right central incisor (tooth 11) was electrically stimulated at an intensity of 3x the individual threshold. The stimulation period lasted 90 sec. The stimulator featured a built-in circuit for measuring electrode resistance. The resistances of the stimulated teeth were monitored throughout the experiment in monopolar coupling, and they ranged from 2 to 4 M Ω .

GCF Samples

GCF samples were collected from the mesiobuccal and distobuccal aspects of each tooth prior to tooth stimulation, during stimulation, and after stimulation ended. Gingival tissue around the stimulated and non-stimulated control teeth underwent careful periodontal examination, which included measurement of plaque index, gingival bleeding, probing depths, and a panoramic radiographic examination. None of the volunteers had used antibiotics within the preceding month. To avoid blood contamination and possible stimulation of GCF flow during clinical measurements (probing, etc.), we collected GCF samples prior to other clinical recordings. The sampling site was isolated with cotton rolls, and supragingival plaque was carefully removed. The region was dried with a gentle air stream, and GCF was then collected by means of standardized filter paper strips. The strip was placed into the crevice until mild resistance was felt, and left there for 30 sec. After that, the strip was immediately placed in a polypropylene tube. We measured the GCF volume with a standardized weighing method (Mettler AJ 100/GWB) by weighing the polypropylene tube with the standardized filter paper strip inside, before and immediately after the sample collection. Thereafter, each strip was eluted into 300 μ L of 20 mM phosphate buffer, pH 6.0, containing 0.15 M NaCl and 0.1% Tween 20 (Apajalahti *et al.*, 2003). The eluates were stored at -20°C prior to analysis. Our recent study (Mäntylä *et al.*, 2003) has showed the average comparative GCF MMP-8 levels in adult periodontitis to be about 2500 μ g/L, in gingivitis, 750 μ g/L, and in healthy sites, 100 μ g/L.

Western Immunoblot and Immunofluorometric Assay (IFMA)

We analyzed the molecular forms of MMP-8 in GCF from the stimulated and control teeth by the Western immunoblot method, with specific polyclonal antibody for MMP-8 used at 2 μ g/mL final concentrations, and by quantitated computer image scanning as previously described (Hanemaaijer *et al.*, 1997; Lindy *et al.*, 1997; Kiili *et al.*, 2002; Prikk *et al.*, 2002; Apajalahti *et al.*, 2003). Human PMN and rheumatoid synovial culture media (Hanemaaijer *et al.*, 1997; Lindy *et al.*, 1997; Kiili *et al.*, 2002) served as positive controls for MMP-8.

MMP-8 concentrations in the GCF were determined by a time-resolved immunofluorometric assay (IFMA), and the amounts of monoclonal antibodies 8708 and 8706 for MMP-8 were 1.5 μ g and 0.5 μ g *per* assay, respectively (Hanemaaijer *et al.*, 1997; Apajalahti *et al.*, 2003; Mäntylä *et al.*, 2003).

Course of the Experiments

The subjects were comfortably seated in a dental chair. The investigation consisted of two sessions with a one-week interval. In the first session, eight human volunteers underwent painful tooth pulp stimulation (3x pain threshold) of the upper right central incisor. The subjective pain levels were evaluated by a visual

analogue scale, VAS (0 = no pain, 100 = the worst imaginable pain intensity). A total of 8 GCF samples was collected from the upper right central incisor (tooth 11 = stimulated tooth), from the upper left lateral incisor (tooth 22), and from the lower right central incisor (tooth 41). Samples 1 and 2 were taken before the stimulation began, and their average value served as a baseline. Samples 3 and 4 were taken during stimulation, followed by samples 5 to 8, taken after the end of tooth stimulation. In the second control session, GCF samples were similarly collected, without tooth stimulation, from six subjects who had participated in the first session. During tests, each subject's heart rate (HR) and mean arterial blood pressure (MAP) responses were semi-automatically recorded (Omron, Digital blood pressure monitor, HEM-705C, Osaka, Japan) from the left upper arm at the same time as the GCF samples were taken. HR and MAP measurements served to clarify whether pulpal pain may have induced some systemic stress reactions, as indicated by possible changes in cardiovascular parameters (Kempainen *et al.*, 2001).

Data Analysis

MMP-8 values were normalized due to large inter-individual variability. The grand mean of all MMP-8 values from each measurement site was calculated separately. For ongoing statistical analysis, the means of baseline, stimulation, 4 min after, and 8 min after the end of stimulation values were extracted and normalized with this grand mean (percent change as compared with grand mean). A Friedman non-parametric ANOVA was then used to compare baseline, stimulation, and post-stimulation periods. In case of significance, a Wilcoxon's matched-pair test was performed for *post hoc* comparisons. A p-value of less than 0.05 was considered significant.

RESULTS

Clinical evaluation of the parameters and radiographs of control sites and of sites exposed to painful stimulation demonstrated that gingival and periodontal health was excellent. During experiments, painful tooth stimulation had no effect on GCF flow at any site (data not shown).

The average intensity of this stimulation was 47 ± 2.2 μ A ($\pm$ SEM, n = 8), and the respective average pain magnitude estimate on VAS scores was 74.4 ± 3.8 ($\pm$ SEM, n = 8).

Stimulation of tooth 11 significantly raised MMP-8 levels (IFMA analysis) in adjacent GCF. These pulpal-pain-evoked elevations in MMP-8 levels occurred during stimulation and remained elevated several minutes after the end of stimulation. Simultaneously, no marked changes in GCF MMP-8 levels could be detected at tooth 22 or tooth 41 (Fig. 1). The control session without stimulation showed that the repeated measurements themselves did not modulate GCF MMP-8 levels (data not shown). The elevated MMP-8 levels in the GCF of the stimulated tooth were clearly lower than those of the gingivitis and periodontitis sites in our earlier study (Mäntylä *et al.*, 2003).

The representative Western immunoblot for molecular forms and degree of activation of MMP-8 of the stimulated incisor (Fig. 2) shows that the samples contained bands at 60 to 80 kDa, corresponding to PMN-type active and pro-enzymes, and from 45 to 55 kDa, corresponding to mesenchymal-type (non-PMN) active and pro-enzymes. We found that the PMN-type isoform was slightly elevated during tooth stimulation (lane 5), and the non-PMN-type MMP-8 was elevated after the end of

Figure 1. Matrix metalloproteinase (MMP)-8 level in gingival crevicular fluid (GCF) and its spatial relation to painful tooth stimulation. Average changes (mean $\pm$ SEM, $n = 8$) of MMP-8 levels in GCF of different teeth during painful stimulation of tooth 11. Shown are the relative changes from baseline during stimulation, 4 min, and 8 min after the end of stimulation. The stars mark significant differences as compared with baseline (Wilcoxon matched-pairs). Data for the Fig. were counted from quantitative IFMA values normalized to baseline (baseline = 100%).

tooth stimulation (lanes 6, 7). No marked changes appeared in blots for MMP-8 of the non-stimulated teeth (data not shown).

Tooth stimulation significantly elevated GCF MMP-8 levels at the stimulated tooth. These levels remained elevated 4 and 8 min after the end of stimulation. Tooth stimulation had no marked effect on systemic HR or MAP values (Table).

DISCUSSION

In the present study, high-intensity stimulation of the upper right incisor induced a local elevation in the GCF MMP-8 levels of the stimulated tooth. In contrast, the GCF MMP-8 levels of the contralateral upper incisor or ipsilateral lower incisor remained unchanged. These results indicate that painful tooth stimulation can induce local inflammatory responses with enhanced proteolytic potential in the very adjacent gingival tissues, and that pulpal pain can contribute to the local regulation of MMP-8 levels in GCF.

MMPs are known to be produced by various immune-effector cells, such as neutrophils, monocytes/macrophages, and fibroblast-like cells (Uitto *et al.*, 2003; Sorsa *et al.*, 2004), often influenced by the pro-inflammatory neuropeptides released from the closely apposed sensory nerves in various tissues (McGillis and Fernandez, 1999), including gingiva (Luthman *et al.*, 1989). Animal studies have shown the existence of branched nerves innervating both intrapulpal and periodontal tissues (Foster and Robinson, 1994). Activation of nociceptive afferents induces a local axon-reflex-mediated neurogenic inflammatory reaction (Wårdell *et al.*,

Figure 2. A representative example of changes in Western immunoblots for molecular forms of MMP-8 from the stimulated tooth. The GCF samples contained bands at 60-80 kDa, corresponding to PMN-type active and pro-enzymes, and at 45-55 kDa, corresponding to mesenchymal-type (non-PMN) active and pro-enzymes. PMN-type isoform was slightly elevated during tooth stimulation (lane 5), and non-PMN-type MMP-8 was elevated after the end of tooth stimulation (lanes 6, 7). PMN indicates PMN-type MMP-8 (lane 1), and F indicates mesenchymal-type MMP-8 (lane 8) from cultured human rheumatoid synovial fibroblasts. Lanes 2 to 7 represent molecular forms of MMP-8 in baseline prior to (pre; lanes 2 and 3), during (stim; lanes 4 and 5), 4 min after (post; lane 6), and 8 min after (post, lane 7) tooth stimulation. Mobilities of molecular-weight markers appear at the left.

1993), which results from the release of pro-inflammatory neuropeptides such as substance P (SP) and calcitonin-gene-related-peptide (CGRP) from the peripheral nerve terminals. Interestingly, recent clinical findings in patients suffering from pulpal pain and inflammation confirm that pulpal pain can cause an axon-reflex-mediated elevation of these pro-inflammatory mediators in the GCF of the painful tooth (Awawdeh *et al.*, 2002a). Thus, this pulpal stimulation-evoked increase in the GCF MMP-8 levels of the stimulated tooth (described above) could result from a local neurogenic, possibly axon-reflex-mediated, inflammatory mechanism.

Evidence suggests that the release of neuropeptides from the peripheral terminals of afferent nerve fibers can modulate inflammatory mediators, cytokines, and proteolytic enzymes in inflammatory diseases (Scott *et al.*, 1994). SP and related neuropeptides exert potent and extensive pro-inflammatory actions, such as increased capillary permeability, vasodilatation, prostaglandin E_2 , MMP, interleukin (IL)-1, and tumor necrosis factor (TNF)- α secretion by resident cells at the sites of inflammation (Scott *et al.*, 1994). IL-1, TNF- α , and PGE₂ can trigger neutrophils, gingival fibroblasts, and epithelial cells to express MMPs, including MMP-8 (Uitto *et*

Table. Pulpal Pain Elevated Local GCF MMP-8 Levels^a But Had no Effect on Systemic HR^b or MAP^c

	Baseline	Stimulation	4 Min after end of stimulation	
GCF MMP-8 of the stimulated tooth ($\mu\text{g/L}$)	23.2 $\pm$ 6.7	44.6 $\pm$ 12.2*	56.5 $\pm$ 16.4*	40.2 $\pm$ 20.4
Heart rate (beats/min)	67 $\pm$ 3.2	65 $\pm$ 2.5	65 $\pm$ 2.3	66 $\pm$ 3.6
Mean arterial pressure (mmHg)	90 $\pm$ 2.4	92 $\pm$ 1.6	89 $\pm$ 3.2	90 $\pm$ 2.3

^a Quantitative GCF MMP-8 concentration based on IFMA data.

^b Heart rate (HR).

^c Mean arterial pressure (MAP). An asterisk (*) indicates the significant difference as compared with baseline (Wilcoxon matched-pairs). All data are presented as average values (mean $\pm$ SEM, $n = 8$) over all subjects.

al., 2003; Sorsa *et al.*, 2004). Overall, neuropeptides, either directly or through action on cytokines, prostaglandins, and TNF- α , can induce the secretion of MMP-8 in the periodontium (Scott *et al.*, 1994; Hanemaaijer *et al.*, 1997; Tervahartiala *et al.*, 2001; Uitto *et al.*, 2003; Sorsa *et al.*, 2004).

In this study, the resistances of stimulated teeth varied between subjects from 2 to 4 M Ω , indicating that no short-circuiting to periodontal tissues occurred (Närhi *et al.*, 1982). Moreover, electrophysiological evidence indicates that the maximal strength (below 60 μ A) of the electrical current used in this study for dental stimulation, even when applied to the periodontium, does not activate extrapulpal nerve fibers (Närhi *et al.*, 1982). These findings indicate that the activation of intrapulpal nociceptive afferent fibers triggered MMP-8 changes in GCF.

Clinical evidence indicates that, in comparison with healthy teeth, teeth with symptomatic pulpitis have increased concentrations of pro-inflammatory neuropeptides in pulp tissue and in GCF (Awawdeh *et al.*, 2002a,b), and of MMP-8 (Wahlgren *et al.*, 2002) in pulp tissue of these teeth. Furthermore, a majority of orthodontic patients report tooth pain at 4 to 24 hrs after the insertion of fixed appliances (Scheurer *et al.*, 1996); this pain is associated with elevated GCF MMP-8 levels from periodontally healthy teeth (Apajalahti *et al.*, 2003). Overall, such elevated levels of pro-inflammatory neuropeptides and MMP-8 in painful teeth occur both in pulp tissue and in adjacent GCF.

Pulpal pain and inflammation lead to an increase in intrapulpal pressure (Byers and Närhi, 1999). Thus, increased fluid flow from the root canal system through the apical foramen and dentinal tubules may cause delivery of MMP-8 to periodontal ligament space. In our study, the subjects' gingival health was excellent and without gingival recession, which contradicts the idea that increased GCF MMP-8 levels resulted from direct migration from tooth pulp to GCF.

Stress has been shown to modulate some periodontitis-relevant immune parameters in GCF (Deinzer *et al.*, 1999). Since pulpal pain evoked only local elevations in GCF MMP-8 levels and caused no marked modulations in systemic cardiovascular parameters, systemic stress mechanisms probably did not significantly contribute to the present results.

The present investigation indicates that experimental pulpal pain can produce local elevations in GCF levels of the potent host-tissue-destructive protease, MMP-8. Analysis of these data supports the possibility of a local neurogenic spread of inflammation from intrapulpal to surrounding periodontal tissues. Moreover, this pulp-based neurogenic process may reflect the phenomenon observed in normal tissue turnover, and, perhaps, predispose such sites to the progression of periodontal destruction (Sorsa *et al.*, 2004). Alternatively, depending on the role of MMP-8, this reaction may also be, at least in part, anti-inflammatory or defensive (Owen *et al.*, 2004).

ACKNOWLEDGMENTS

This study was financially supported by the Academy of Finland, the Else and Wilhelm Stockmann Foundation, the Finnish Cultural Foundation, the Finnish Dental Society Apollonia, the Finnish Female Dentists' Association, the Helsinki University Research Funds, the HUCH-EVO (TI020Y0002 and TYH4113) Grants, and the DAAD.

REFERENCES

- Apajalahti S, Sorsa T, Railavo S, Ingman T (2003). The *in vivo* levels of matrix metalloproteinase-1 and -8 in gingival crevicular fluid during initial orthodontic tooth movement. *J Dent Res* 82:1018-1022.
- Awawdeh LA, Lundy FT, Linden GJ, Shaw C, Kennedy JG, Lamey PJ (2002a). Quantitative analysis of substance P, neurokinin A and calcitonin gene-related peptide in gingival crevicular fluid associated with painful human teeth. *Eur J Oral Sci* 110:185-191.
- Awawdeh L, Lundy FT, Shaw C, Lamey PJ, Linden GJ, Kennedy JG (2002b). Quantitative analysis of substance P, neurokinin A and calcitonin gene-related peptide in pulp tissue from painful and healthy human teeth. *Int Endod J* 35:30-36.
- Byers MR, Närhi ML (1999). Dental injury models: experimental tools for understanding neuroinflammatory interactions and polymodal nociceptor functions. *Crit Rev Oral Biol Med* 10:4-39.
- Deinzer R, Förster P, Fuck L, Herforth A, Stiller-Winkler R, Idel H (1999). Increase of crevicular interleukin 1beta under academic stress at experimental gingivitis sites and at sites of perfect oral hygiene. *J Clin Periodontol* 26:1-8.
- Fazekas A, Vindisch K, Posch E, Gyorfi A (1990). Experimentally-induced neurogenic inflammation in the rat oral mucosa. *J Periodontal Res* 25:276-282.
- Foster E, Robinson PP (1994). A study of branched pulpal axons in ferrets with supplemental teeth. *Arch Oral Biol* 39:1003-1005.
- Hanemaaijer R, Sorsa T, Kontinen YT, Ding Y, Sutinen M, Visser H, *et al.* (1997). Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. *J Biol Chem* 272:31504-31509.
- Holzer P (1988). Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neuroscience* 24:739-768.
- Kemppainen P, Pertovaara A, Huopaniemi T, Johansson G, Karonen SL (1985). Modification of dental pain and cutaneous thermal skin sensitivity by physical exercise in man. *Brain Res* 360:33-40.
- Kemppainen P, Forster C, Handwerker HO (2001). The importance of stimulus site and intensity in differences of pain-induced vascular reflexes in human orofacial regions. *Pain* 91:331-338.
- Kemppainen P, Avellan NL, Handwerker HO, Forster C (2003). Differences between tooth stimulation and capsaicin-induced neurogenic vasodilatation in human gingiva. *J Dent Res* 82:303-307.
- Kiili M, Cox SW, Chen HY, Wahlgren J, Maisi P, Eley BM, *et al.* (2002). Collagenase-2 (MMP-8) and collagenase-3 (MMP-13) in adult periodontitis: molecular forms and levels in gingival crevicular fluid and immunolocalisation in gingival tissue. *J Clin Periodontol* 29:224-232; erratum in *J Clin Periodontol* 31:149, 2004.
- Lindy O, Kontinen YT, Sorsa T, Ding Y, Santavirta S, Ceponis A, *et al.* (1997). Matrix metalloproteinase 13 (collagenase 3) in human rheumatoid synovium. *Arthritis Rheum* 40:1391-1399.
- Listgarten MA (1987). Nature of periodontal diseases: pathogenic mechanisms. *J Periodontal Res* 22:172-178.
- Luthman J, Friskopp J, Dahllof G, Ahlstrom U, Sjöström L, Johansson O (1989). Immunohistochemical study of neurochemical markers in gingiva obtained from periodontitis-affected sites. *J Periodontal Res* 24:267-278.
- Mäntylä P, Stenman M, Kinane DF, Tikanoja S, Luoto H, Salo T, *et al.* (2003). Gingival crevicular fluid collagenase-2 (MMP-8) test stick

- for chair-side monitoring of periodontitis. *J Periodontol Res* 38:436-439.
- McGillis JP, Fernandez S (1999). Sensory neuropeptides, neurogenic inflammation and inflammatory cells. In: Pain and neurogenic inflammation. Brain SD, Moore PK, editors. Basel: Birkhauser, pp. 115-135.
- Närhi M, Virtanen A, Huopaniemi T, Hirvonen T (1982). Conduction velocities of single pulp nerve fibre units in the cat. *Acta Physiol Scand* 116:209-213.
- Owen CA, Hu Z, Lopez-Otin C, Shapiro SD (2004). Membrane-bound matrix metalloproteinase-8 on activated polymorphonuclear cells is a potent tissue inhibitor of metalloproteinase-resistant collagenase and serpinase. *J Immunol* 172:7791-7803.
- Prikk K, Maisi P, Pirilä E, Reintam MA, Salo T, Sorsa T, *et al.* (2002). Airway obstruction correlates with collagenase-2 (MMP-8) expression and activation in bronchial asthma. *Lab Invest* 82:1535-1545.
- Scheurer PA, Firestone AR, Burgin WB (1996). Perception of pain as a result of orthodontic treatment with fixed appliances. *Eur J Orthod* 18:349-357.
- Scott DT, Lam FY, Ferrell WR (1994). Acute joint inflammation—mechanisms and mediators. *Gen Pharmacol* 25:1285-1296.
- Sorsa T, Uitto VJ, Suomalainen K, Vauhkonen M, Lindy S (1988). Comparison of interstitial collagenases from human gingiva, sulcular fluid and polymorphonuclear leukocytes. *J Periodontol Res* 23:386-393.
- Sorsa T, Tjäderhane L, Salo T (2004). Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis* 10:311-318.
- Stead RH, Dixon MF, Bramwell NH, Riddell RH, Bienenstock J (1989). Mast cells are closely apposed to nerves in the human gastrointestinal mucosa. *Gastroenterology* 97:575-585.
- Tervahartiala T, Koski H, Xu JW, Häyrynen-Immonen R, Hietanen J, Sorsa T, *et al.* (2001). Tumor necrosis factor- α and its receptors, p55 and p75, in gingiva of adult periodontitis. *J Dent Res* 80:1535-1539; *erratum in J Dent Res* 80:1684.
- Uitto VJ, Overall C, McCulloch C (2003). Proteolytic host cell enzymes in gingival crevice fluid. *Periodontol* 2000 31:77-104.
- Wahlgren J, Salo T, Teronen O, Luoto H, Sorsa T, Tjäderhane L (2002). Matrix metalloproteinase-8 (MMP-8) in pulpal and periapical inflammation and periapical root-canal exudates. *Int Endod J* 35:897-904.
- Wårdell K, Naver HK, Nilsson GE, Wallin BG (1993). The cutaneous vascular axon reflex in humans characterized by laser Doppler perfusion imaging. *J Physiol* 460:185-199.