Objective. The aim of this study was to investigate levels of matrix metalloproteinase-8 (MMP-8) and substance P (SP) in gingival crevicular fluid (GCF) during root canal treatment (RCT) of nonvital teeth.

Study design. Patients scheduled for nonsurgical RCT were prospectively selected; all patients provided informed consent. GCF samples were collected from teeth scheduled for RCT and their contralateral teeth across 3 different time periods. MMP-8 and SP levels were measured using enzyme-linked immunosorbent assay (ELISA). Data were analyzed using a mixed model analysis and the Pearson correlation analysis.

Results. Patients' subjective pain levels were significantly related to both MMP-8 and SP levels. MMP-8 and SP levels in GCF were decreased during RCT, and they showed a positive correlation with each other (P < .05).

Conclusions. This study demonstrated that periradicular inflammation of endodontic origin can elevate SP and MMP-8 levels in GCF. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:548-554)

Matrix metalloproteinases (MMPs) are upregulated during inflammation and related to the breakdown of extracellular matrix. In endodontic research, MMPs have been detected in inflamed dental pulp, periapical tissue, and cystic fluid. Subtypes MMP-1 and MMP-8 have been the focus of previous periodontal studies because these collagenases may contribute to physiological and pathologic collagen degradation in gingival tissues. MMP-8 is thought to be produced by not only polymorphonuclear (PMN) cells, but also non-PMN lineage cells, such as fibroblasts, endothelial cells, plasma cells, and odontoblasts. Previous studies have demonstrated that increased GCF levels of MMP-8 are associated with more severe chronic periodontitis, and these levels decreased after successful periodontal therapy. Because endodontic pathoses may also contribute to periodontal destruction or inflammation, MMPs and/or cytokines in GCF may be elevated, such as they are with periodontal diseases. In one study, when painful tooth stimulation was applied, the levels of MMP-8 and SP in GCF were elevated. The initial high level of MMP-8 in periapical exudate samples decreased during root canal treatment (RCT), which indirectly showed that MMP-8 might have a role in pulpal and periapical inflammation. Based on this background information, this study investigated the levels of MMP-8 and SP in GCF during RCT and the potential relationships among patients' pain, clinical signs and symptoms, and these inflammatory mediator levels.
MATERIAL AND METHODS

Endodontic patients

GCF samples were obtained from 35 teeth from 25 patients (13 males and 12 females, average age 43.8 years) who visited the Department of Conservative Dentistry, Gangnam Severance Dental Hospital, Yonsei University, Seoul, Korea, for RCT between September 24, 2008, and September 24, 2009. Teeth selected for the study had been diagnosed as necrotic pulp or previously initiated therapy with symptomatic or asymptomatic periradicular inflammation or abscess. The study was performed after obtaining informed consent from the patients and was approved by the Institutional Review Board for Clinical Research of Gangnam Severance Hospital, Yonsei University (3-2008-0118). Also, the Helsinki Declaration was read and the guidelines were followed. None of the patients had contributory medical histories; none of the patients had taken any antibiotics or pain medicine within 1 week before or during the treatment period. Patients were excluded from the study if they required antibiotic prophylaxis or had uncontrolled diabetes, generalized periodontitis, or teeth with pocket-probing depths of more than 3 mm.

GCF sampling

GCF collection was performed during 3 consecutive appointments with maximum 3-week intervals between the appointments: before initiating access into the root canal (period 1), before canal irrigation at the second visit (period 2), and before canal filling at the third visit (period 3).

To avoid blood contamination, GCF collection was performed before any other clinical tests. The sampling sites were selected from the buccal aspects of the mesial and distal surfaces at the interproximal sites. The contralateral tooth was selected as a control for each sample.

After the tooth was isolated with cotton rolls, supragingival plaque was carefully removed, and the sulcus was dried by blowing gentle air. A sterilized, medium-sized paper point (Diadent Korea, Seoul, Korea) was inserted into the gingival sulcus and left in place for 30 seconds. A second paper point was inserted, and the same procedure was repeated. In total, 4 paper points absorbing GCF were placed into a 1.5-mL Eppendorf tube containing 100 μL of Tris-HCl buffer, pH 7.5, with 0.15 M NaCl and 1 mM CaCl2; the tubes were placed on a shaker at room temperature for 3 hours and then stored at −70°C.

Patients’ subjective symptoms and clinical examination

Each patient’s pain level was recorded using the Visual Analogue Scale (VAS; 0 to 10 scale, 0: no pain, 10: extremely severe pain). Several other evaluations were completed after GCF sampling: percussion test (0: no response, +: slight pain, ++: moderate pain, +++: severe pain), mobility test (0: immobile, 1: slight horizontal movement, 2: moderate to severe horizontal movement, 3: vertical movement), and observation of sinus tract (0: none, 1: sinus tract observed). In addition to these clinical observations, periodontal pocket probing, a bite test, thermal tests, and radiographic examinations were completed as routine procedures.

RCT procedure

Primary access to the pulp chamber was completed with high-speed burs under rubber dam isolation. Canals were enlarged minimally up to #35 using Gates Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland), hand K- and H-files (Sybron, Endo Co., Orange, CA), ProTaper S1/S2 (Dentsply Maillefer), and the ProFile system (Dentsply Maillefer). Three percent sodium hypochlorite was used as a routine irrigant. The access cavity was temporized with caviton (GC Co., Tokyo, Japan).

After 2 weeks, the tooth was isolated with a rubber dam, and the temporary filling was removed. A canal(s) was (were) irrigated with 3% sodium hypochlorite.

After 2 weeks, root canal obturation was performed by a continuous wave of the condensation technique using System B (Sybron, Endo Co.) and Duo-beta (Phildent, Seoul, Korea).

MMP-8 and SP analyses

Quantification of MMP-8 and SP was completed with a human MMP-8 (total) kit (Quantikine, R&D Systems Inc., Minneapolis, MN) and a competitive enzyme immunoassay kit (Parameter, R&D Systems Inc.) according to the manufacturers’ instructions, respectively. Fifty microliters of each sample, as well as standards and controls, was pipetted into 96-well plates coated with specific antibodies. All samples were analyzed in duplicate. The absorbency of each sample at 450 nm was read by a spectrophotometer (enzyme-linked immunosorbent assay [ELISA] reader, Molecular Devices, Inc/E-max, Menlo Park, CA). The data were expressed as the total amount (ng or pg) per sample.

Statistical analysis

A mixed model analysis for repeated measures was used. To assess each patient’s pain level related to the expression of MMP-8 and SP, a mixed model analysis was conducted with the between-subjects factor of pain level (VAS 0-10) entered as the within-
subjects factor. To evaluate the possible effects of various clinical findings, such as the percussion test, mobility test, and sinus tract observation, the statistical analysis was performed in the same manner as for pain level. The Pearson correlation coefficient was calculated to determine if MMP-8 and SP had any correlation with each other during any time period. A $P$ value less than .05 was considered statistically significant. SAS (version 9.1.3, SAS Institute, Inc., Cary, NC) was used for statistical analysis.

RESULTS

MMP-8 and SP level

MMP-8 and SP levels from each experimental individual are shown in Fig. 1. The variation among individuals, depending on the time periods, was more obvious in the experimental groups than in the controls. Both MMP-8 and SP levels were decreased during RCT, and the differences observed in the MMP-8 and SP levels among individuals from 3 consecutive time periods were statistically significant ($P < .0001$, Fig. 2). Because the amount of MMP-8 and SP from the contralateral tooth was measured 3 times, estimates and standard errors were calculated by adjusting control values using a mixed model (Fig. 3).

The correlation between the levels of MMP-8 and SP

At each time period, the amounts of MMP-8 and SP showed a positive correlation with each other. At the
first time period (before treatment), the levels of MMP-8 and SP showed a strong positive correlation (Pearson correlation coefficient \( \rho = 0.50346 \)), which was statistically significant \( (P = 0.0020) \).

The effect of patients’ subjective pain on the levels of MMP-8 and SP
A significant relationship \( (P = 0.0004) \) was found between patients’ pain levels and the amount of MMP-8 in GCF. Also, patients’ pain levels were significantly related to the amount of SP in GCF \( (P = 0.0425) \).

The effect of clinical findings on the level of MMP-8 and SP
The percussion and tooth mobility tests showed a strong association with MMP-8 levels \( (P = 0.0006 \text{ and } P = 0.01863, \text{ respectively}) \). However, there was no statistical significance in the SP levels related to percussion, tooth mobility, or the existence of a sinus tract (Table I).

DISCUSSION
In the present study, we investigated the MMP-8 and SP levels in GCF during RCT on a painful nonvital tooth. Significantly elevated levels of MMP-8 and SP in GCF were found in the painful tooth. In experimental samples, the Pearson correlation analysis revealed a significant correlation between levels of MMP-8 and SP, even though the distribution of SP was more scattered than that of MMP-8.

In gingival and periapical tissues, sensory nerves are closely related to immuno-effector cells.\(^\text{17}\) Experimental tooth pain, as well as painful pulpitis, elevated the SP and MMP-8 levels in GCF.\(^\text{2,15,18}\) The results from our study were consistent with previous studies that showed a possible role of neurogenic inflammation in the progression of periradicular inflammation. Awawdeh et al.\(^\text{2}\) measured the amount of neuropeptides in GCF from teeth that had been diagnosed with irreversible pulpitis and discussed the possible pathways of increasing SP and NKA in GCF. One possibility that had been cited was an...
increase in the rate of fluid flow out of the root canal system; the fluid carried released neuropeptides. The MMP-8 levels that were measured in the root canal exudates indicated that the MMP-8 found in exudates originated from the periapical inflammation rather than pulp tissue. Because all the samples in the present study consisted of teeth with necrotic pulp or previously initiated treatment, the source of elevated MMP-8 and SP may have been nerves and tissues in the periodontium. Among all MMPs, MMP-8 is the most destructive and is abundant in periodontally diseased gingiva and GCF.

The GCF volume of healthy control teeth was significantly lower than that of samples with gingivitis or periodontitis. GCF volume was increased in diseased sites, possibly resulting in the decreased cytokine concentrations. Total amounts of cytokines in GCF per sampling time were suggested to investigate the relative GCF constituent activity from different studies. Therefore, the present study used data based on the total amount of MMP-8 and SP per sample, and the same amount of time was used to collect GCF from each site.

There were variations in the levels of MMP-8 and SP, which indicated substantial differences among the individuals. Therefore, the changes observed within an individual were considered more important than the absolute value of each measurement. A mixed model statistical analysis was used to investigate longitudinal data from the same individual. The data were adjusted for each control value to investigate the effects of a significant lower than that of samples with gingivitis or periodontitis. GCF volume was increased in diseased sites, possibly resulting in the decreased cytokine concentrations. Total amounts of cytokines in GCF per sampling time were suggested to investigate the relative GCF constituent activity from different studies. Therefore, the present study used data based on the total amount of MMP-8 and SP per sample, and the same amount of time was used to collect GCF from each site.

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patient’s subjective pain level, responses to the percussion and bite tests, and the existence of a sinus tract on and bite tests, and the existence of a sinus tract on and bite tests, and the existence of a sinus tract on and bite tests, and the existence of a sinus tract on and bite tests, and the existence of a sinus tract on and bite tests, and the existence of a sinus tract on and bite tests, and the existence of a sinus tract on and bite tests, and the existence of a sinus tract on and bite tests, and the existence of a sinus tract.

We selected the contralateral tooth as a control because branches of sensory nerves can innervate both adjacent pulps and surrounding periodontal tissues.

Previous studies used commercially available paper strips (Periopaper, Proflow Inc., Amityville, NY) or an intracrevicular washing method. In this study, sterilized, medium-sized paper points were used instead of paper strips. Wahlgren et al. also used paper points for collecting root canal exudates to measure MMP-8 levels. The consistent longitudinal data for control sites indicate the appropriate use of paper points as collecting devices for GCF.

Different methodologies have been used to measure the amount of MMP-8 in GCF. Munjal et al. investigated the levels of MMP-8 in GCF using a time-resolved immunofluorometric assay (IFMA), ELISA, and a dentoAnalyzer device; the test results were comparable.Recently, other evaluations have shown that MMP-8 levels in GCF that were measured using IFMA, a chair-side device, and a dentoAnalyzer were consistent with each other; however, levels measured using ELISA did not agree with the other results. Sorsa et al. reported that their results might come from different MMP-8 antibodies used in ELISA. Only the total amount of MMP-8 is measured with ELISA, which might have been a limitation when interpreting the results of this present study. Active forms of MMP-8 in GCF are related to progressive periodontitis, whereas the latent form is primarily found in mild gingivitis.

Chair-side tests that detect MMP-8 in GCF have been developed and used for diagnosing and monitoring periodontal diseases. These types of devices have been proposed for use in endodontics as future diagnostic tools. Despite a clear decrease in GCF, MMP-8, and SP levels during RCT, the presence or slight increase of these proteins was observed in a small number of samples. Interestingly, these samples were primarily from patients who expressed persistent discomfort throughout the appointments.

The present investigation found that periradicular inflammation can elevate SP and MMP-8 levels in GCF; this supports the possibility of neurogenic spread of inflammation from periapical tissue to surrounding periodontal tissues. Pain appears to be the most reliable factor in determining MMP-8 and SP levels in GCF during RCT.

REFERENCES


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