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Decreased levels of matrix metalloproteinase-2 in root-canal exudates during root canal treatment

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ABSTRACT

Objective: To determine the matrix metalloproteinase-2 (MMP-2) levels in root-canal exudates from teeth undergoing root-canal treatment.

Material and methods: The root-canal exudates from six teeth with normal pulp and periradicular tissues that required intentional root canal treatment for prosthodontic reasons and from twelve teeth with pulp necrosis and asymptomatic apical periodontitis (AAP) were sampled with paper points for bacterial culture and aspirated for the detection of proMMP-2 and active MMP-2 by gelatin zymography and the quantification of MMP-2 levels by ELISA.

Results: By gelatin zymography, both proMMP-2 and active MMP-2 were detected in the first collection of root-canal exudates from teeth with pulp necrosis and AAP, but not from teeth with normal pulp, and their levels gradually decreased and disappeared at the last collection. Consistently, ELISA demonstrated a significant decrease in MMP-2 levels in the root-canal exudates of teeth with pulp necrosis and AAP following root canal procedures ($p < 0.05$). Furthermore, the MMP-2 levels were significantly lower in the negative bacterial culture than those in the positive bacterial culture ($p < 0.001$).

Conclusions: The levels of MMP-2 in root-canal exudates from teeth with pulp necrosis and AAP were gradually reduced during root canal procedures. Future studies are required to determine if MMP-2 levels may be used as a biomolecule for the healing of apical lesions, similar to the clinical application of MMP-8 as a biomarker.

1. Introduction

Pulpal infection and periradicular lesions are caused by oral microorganisms (Kakehashi, Stanley, & Fitzgerald, 1965; M & ller, Fabricius, Dahlén, Ohman, & Heyden, 1981). In the process, invading microbes activate human immune responses, including both protective and destructive reactions that lead to pulpitis and eventually total pulp necrosis. If untreated, destruction of the bone surrounding the root apex of the affected tooth ensues (Nair, 1997). In order to prevent or manage the periradicular lesions, root-canal treatment is indicated. A major aim of this treatment is to eliminate bacteria and their byproducts from the root-canal system through mechanical cleansing and chemical irrigation (Bystrom, Happonen, Sjogren, & Sundqvist, 1987; Nair, 2004).

A bacterial culture from the root-canal exudate has been regarded in the past by some clinicians and researchers to predict the successful outcome of treatment. However, bacterial culture is a time-consuming process and is costly if anaerobic culture is required to grow fastidious bacteria (Sathorn, Parashos, & Messer, 2007). Therefore, host-derived biomolecules from apical tissues and root-canal exudates would be an alternative for prediction of treatment outcomes.

In the pathogenesis and the healing processes of periradicular lesions, a number of biomolecules are involved, such as the family members of Zn^{2+}-dependent matrix metalloproteinases (MMPs) (Marton & Kiss, 2000; Nair, 2004). MMPs are capable of degrading extracellular matrix and basement membrane constituents (Birkedal-Hansen et al., 1993; Visse & Nagase, 2003). MMPs play an important role in several physiological phenomena, as well as in pathological conditions, such as atherosclerosis, rheumatoid arthritis, recurrent aphthous ulcers, periodontitis and apical periodontitis (Hannas, Pereira, Granjeiro, & Tjaderhane, 2007; Makela, Salo, Uitto, & Larjava,

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1994; Malemud, 2006; Mantyla et al., 2003; Sorsa, Tjaderhane, & Salo, 2004; Sousa et al., 2014). In particular, MMP-2, or gelatinase A, is a 72-kDa enzyme known to degrade type IV collagen, a major component found in the basement membrane. This enzyme plays a predominant role in the regulation of angiogenesis and inflammation. MMP-2 is expressed in inflamed pulp tissue and periradicular pathosis, and thus plays an important role in inflammation (Corotti et al., 2009; Letra et al., 2013; Shin, Lee, Baek, & Lim, 2002).

In addition to MMP-2, MMP-8, a 75-kDa neutrophil collagenase, is found in inflamed connective tissue. MMP-8 is activated by autolytic cleavage and its function is to degrade collagen types I, II and III. MMP-8 up-regulation is demonstrated in inflamed pulp and periradicular lesions, and its levels are raised in root-canal exudates (Wahlgren et al., 2002). Therefore, it is suggested that elevated MMP-8 levels could be used to indicate ongoing inflammation in the apical tissue, reflecting the success of root-canal treatment. However, there is a paucity of information regarding the levels of human MMP-2 in root-canal exudates and little is known about the changes in MMP-2 and MMP-8 levels in the root-canal exudates of human teeth undergoing root canal procedures. Therefore, the objective of this study was to detect the presence of MMP-2 in root-canal exudates of human teeth, diagnosed with normal pulp and periradicular tissues or with pulp necrosis and asymptomatic apical periodontitis (AAP). Furthermore, the alterations in MMP-2 and MMP-8 levels were investigated during each treatment visit and compared with bacterial culture results.

2. Materials and methods

2.1. Patients

Eleven patients (age range: 17–69 years old) scheduled for root canal treatment at the Department of Restorative Dentistry and Periodontology, Faculty of Dentistry, Chiang Mai University, were included in this study. A total of twelve teeth, having only one root canal, which were diagnosed with pulp necrosis and AAP from nine patients and six teeth, having only one root canal, which were diagnosed with normal pulp and periradicular tissues that required root-canal therapy for prosthodontic reasons from two patients were selected. Patients with underlying systemic diseases were excluded from this study. Clinical and radiographic examinations were conducted, and the diagnosis was based on clinical symptoms, vitality testing and radiographic interpretation, according to the guidelines of the American Association of Endodontists (Glickman, 2009). Teeth with pulp necrosis and AAP responded negatively to pulp testing, and their radiographic findings showed a radiolucent lesion or loss of lamina dura; patients had no pain on percussion. Teeth with normal pulp responded positively to pulp testing and radiographs showed an intact lamina dura without thickening of periodontal ligament space. All root-canal exudates were collected at baseline and during each treatment visit; all procedures were performed with the approval of the Human Experimentation Committee, Faculty of Dentistry, Chiang Mai University. Informed consent was obtained from all patients prior to sample collection.

2.2. Sample collection

Access to the pulp was achieved using sterile dental burs under dental dam isolation. Radiographic images and an electrical apex locator were used to determine the root-canal length. All root canals were instrumented with a step back technique using master apical files to at least size 40. During instrumentation, the canals were disinfected with 5.25% sodium hypochlorite (NaOCl), and the smear layer was removed by 17% EDTA, using passive ultrasonic irrigation for one minute and a final rinse with 5.25% NaOCl. Root canals were dried with sterile paper points, and a sterile dry cotton pellet dampened with camphorated monochlorophenol (CMP) was placed in the pulp chamber. The access cavity was sealed with Cavit-G (3M ESPE, St. Paul, MN, USA). All patients were recalled within a few days, and the temporary filling and the medicated cotton pellet were removed using an aseptic technique. A 10-µl quantity of sterile buffer, containing 50 mM Tris-HCl pH 7.5, 0.15 M NaCl and 1 mM $CaCl_2$, was added into the root canal for two minutes, and the same volume was aspirated from each root-canal exudate and transferred to an eppendorf tube, containing 40 µl of the same buffer to dilute the exudate fivefold. This dilution was chosen from data obtained in a pilot study that showed two clear and discrete gelatinolytic bands between proMMP-2 and active MMP-2. A sterile paper point was inserted into the canal, left for 30 s, and then put in the tube, containing thioglycollate medium that was further incubated at 37 °C for a standard protocol of bacterial culture. The canal was rinsed with NaOCl and dried. For the teeth with normal pulp, the root canals were filled in this visit, but for those with pulp necrosis and AAP, calcium hydroxide paste, pH 12.5 (Biocalc, Orion, Helsinki, Finland) was placed into the canal and the cavity was sealed. After two weeks, the paste in the root canal was removed and replaced with a sterile dry cotton ball dampened with CMP. A few days later, the procedure for collection of root-canal exudates was repeated and the collections were continued until the bacterial culture was negative. The root canals were filled with gutta-percha and zinc oxide-eugenol sealer (Tubliseal EWT, Kerr Co., Romulus, MI, USA) using cold lateral compaction in the next appointment. All collected root-canal exudates were stored at −80 °C for further analysis.

2.3. Gelatin zymography

Total protein concentrations of all root-canal exudates were determined by the Bio-Rad Protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA), based on the method of Bradford (Bradford, 1976), and expressed in units of mg/ml. An equal amount of total protein from each root-canal exudate was loaded in each lane for detection of MMP-2 activity by gelatin zymography as described previously (Pattamapun, Tiranathanagul, Yongchaitrakul, Kuwatanasuchat, & Pavasant, 2003). Briefly, the root-canal exudates and the conditioned medium collected from primary human periodontal ligament cells, treated with 10 ng/ml of Concanavalin A (Sigma-Aldrich, St. Louis, MO, USA) for 24 h as a positive control for the presence of proMMP-2 and active MMP-2 (Kawagoe, Tsuruga, Oka, Sawa, & Ishikawa, 2013; Overall et al., 2000), were mixed with non-reducing sample buffer, containing 0.5 M Tris-HCl pH 6.8, glycerol, 10% sodium dodecyl sulfate (SDS) without β-mercaptoethanol, and loaded onto 0.1% gelatin-containing SDS-polyacrylamide gel electrophoresis along with the molecular weight markers (Bio-Rad Laboratories) at 100 V for two h. Following electrophoresis, the gels were gently washed with buffer, containing 2.5% Triton-X100 (Sigma-Aldrich) at room temperature three times for 30 min each to remove SDS, and then incubated in activating buffer, containing 0.15 M NaCl, 10 mM $CaCl_2$, 50 mM Tris-HCl pH 7.5, 0.1% Brij-35, at 37 °C for 20 h. The gels were stained with 2.5% Coomassie Brilliant Blue R-250 (Sigma-Aldrich) in 30% methanol and 10% acetic acid, and then de-stained for 30 min in 5% methanol and 7.5% acetic acid. The digitized images of the gelatin zymograms were captured using a LaserJet M1522 MFP Series PCL scanner (Hewlett-Packard, Palo Alto, CA, USA), and the intensities of proMMP-2 and active MMP-2 bands were measured by Scion Image software version beta 4.0.3 (Scion Corporation, Rockville, MA, USA). The ratios of active MMP-2 to proMMP-2 were determined for all root-canal exudate samples.

2.4. Sandwich ELISA for MMP-2 and MMP-8 measurements

The levels of total MMP-2, including proMMP-2 and active MMP-2, and of MMP-8 in the root-canal exudates were determined by the human MMP-2 Immunoassay kit (Quantikine, R & D Systems, Inc., Minneapolis, MN, USA) and by the human total MMP-8 kit (Quantikine, R & D Systems, Inc.), respectively, according to the manufacturer's instructions. Briefly, the root-canal exudates were first diluted with the