Clinical Investigation of Matrix Metalloproteinases, Tissue Inhibitors of Matrix Metalloproteinases, and Matrix Metalloproteinase/Tissue Inhibitors of Matrix Metalloproteinase Complexes and Their Networks in Apical Periodontitis

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Abstract

Introduction: This clinical study investigated the levels of metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) and respective forms (MMP/TIMP complexes) in apical periodontitis to determine their networks in the development of clinical/radiographic features, thus quantifying the levels of endotoxins (lipopolysaccharides) present in primarily infected root canals with apical periodontitis. **Methods:** Twenty primarily infected root canals with apical periodontitis were selected. The presence of pain on palpation, tenderness to percussion, and the size of the radiographic lesion were recorded. The levels of MMPs (MMP-1, -2, and -9), TIMPs (TIMP-1 and -2), and their MMP/TIMP complexes (MMP-1/TIMP-1, MMP-1/TIMP-2, MMP-2/TIMP-1, MMP-2/TIMP-2, MMP9/TIMP-1, and MMP-9/TIMP-2) present in the periapical interstitial fluid were measured using the enzyme-linked immunosorbent assay. The kinetic chromogenic LAL test was used to quantify endotoxins. **Results:** A higher mean level of MMP-9 (968.35 ± 342.00 pg/mL) was followed by MMP-2 (894.00 ± 591.62 pg/mL) and MMP-1 (789.43 ± 342.83 pg/mL). The linear regression analysis revealed a positive association of MMP-1 with both MMP-2 and MMP-9 (all $P < .001$). TIMP-1 (481.79 ± 86.09 pg/mL) (24/24) was found in higher levels than TIMP-2 (206.45 ± 86.09 pg/mL) ($P < .05$), including a positive correlation of MMP-1 with both TIMP-1 and TIMP-2 (all $P < .05$). Higher mean levels of MMP1, -2, and -9 were found in teeth with larger-size radiolucent lesions (>7 mm) compared with smaller ones (≤7 mm) (all $P < .01$). Higher levels of MMP-1 decreased the chance of TTP, whereas MMP-9 (odds ratio = 0.97) increased the chance of pain on percussion (odds ratio = 1.01). Higher levels of endotoxins present in root canals were positively correlated with larger amounts of MMP -9 ($P < .05$). **Conclusions:** MMPs, TIMPs, and their complexes (MMP/TIMP) are involved in apical periodontitis by interacting with complex networks in the development of clinical features and the severity of bone destruction. *(J Endod 2016;42:1082–1088)*

Key Words

Endodontics, endotoxin, infection, root canal

Significance

MMPs, TIMPs, and their complexes play a role in apical periodontitis

Apical periodontitis is a tissue breakdown established in the periapical tissue provoked by the interplay between bacterial infection of the root canal systems and the local immune response (1). Such a local immune response is very complex and involves degradation of the extracellular matrix (ECM) components, the main components of connective tissue (2).

Matrix metalloproteinases (MMPs) (3), a family of zinc- and calcium-dependent endopeptidases, are strongly associated with the breakdown of ECM (4) and play a major role in bone remodeling and resorption (5). According to their target protein, MMPs are divided into several families (6) as follows: collagenase (MMP-1 (7)), gelatinases (MMP-2 (8)), stromelysin (MMP-3 (7)), and matrixin (MMP-9 (9)). Different subtypes of MMPs, including MMP-1, -2, -3, -7, -8, and -9, have been detected in apical periodontitis (3,10–16). Of a clinical concern, elevated MMP levels have been correlated with nonhealing lesions (17, 18).

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2016.04.001

The zinc-dependent endopeptidase activities of the MMPs are inhibited specifically by their intrinsic inhibitors called tissue inhibitors of MMPs (TIMPs) (19–21). TIMPs have been detected in apical periodontitis (16, 22), with TIMP-1 and -2 being multifunction proteins (23). Particularly, TIMP-1 has been suggested as an enhancer or inhibitor of bone resorption depending on whether TIMP-1 concentrations are low or high, respectively (24). Despite the biological activities of MMPs, independent of the MMP-inhibitory activity, the imbalance between the levels of MMPs and TIMPs is critical for ECM remodeling being able to provoke tissue destruction (23). Thus, TIMPs can bind tightly to the active proteinases (MMPs) to form the MMP/TIMP complex (25–27). The MMP/TIMP complex has been speculated by other research fields as a potential biological biomarker for the possible prediction of changes in the course of diseases (28–30).

To help understand the participation of MMPs and TIMPs and their network in apical periodontitis, this clinical study sought to investigate the levels of MMPs and TIMPs and their respective forms (MMP/TIMP complexes) by determining their networks in the development of clinical/radiographic features, thus quantifying the levels of endotoxins (lipopolysaccharides [LPSs]) present in primarily infected root canals with apical periodontitis.

Materials and Methods

Patient Selection

Twenty patients attending São José dos Campos Dental School, São José dos Campos, São Paulo, Brazil, for primary endodontic treatment were included in the present study. The age of patients ranged from 22–45 years old (mean = 35 years, 7 women and 13 men). A detailed dental and general history was obtained from each patient. Those who had received antibiotic treatment during the past 3 months and/or who had any systemic health disorder that might affect the inflammatory response were excluded.

The Human Research Ethics Committee of the São José dos Campos Dental School approved the protocol describing the sample collection for this investigation, and all the volunteer patients signed an informed consent form.

All the single-rooted teeth were maxillary teeth with primary endodontic infection showing the presence of 1 root canal and the absence of periodontal pockets deeper than 4 mm. Teeth that could not be isolated with a rubber dam were also excluded. None of them reported any type of systemic disease. The following clinical/radiographic features were recorded: clinical symptomatology (spontaneous pain) (11/20), pain on palpation (9/20), tenderness to percussion (7/20), and size of radiolucent area >7 mm (11/20).

Sampling Procedures

The files, instruments, and all materials used in this study were treated with Co^{60} gamma radiation (20 kGy for 6 hours) for sterilization and elimination of pre-existing endotoxins (EMBRARAD; Empresa Brasileira de Radiação, Cotia, SP, Brazil) (31). The method used for disinfection of the operative field has been previously described elsewhere (32). Briefly, the teeth were isolated with a rubber dam. The crown and surrounding structures were disinfected with 30% H_2O_2 (volume/volume) for 30 seconds followed by 2.5% sodium hypochlorite (NaOCl) for the same period of time and then inactivated with 5% sodium thiosulfate. The sterility of the external surfaces of the crown was checked by taking a swab sample from the crown surface and streaking it onto blood agar plates, which were then incubated both aerobically and anaerobically.

A 2-stage access cavity preparation was made without the use of water spray but under manual irrigation with sterile/apyrogenic saline solution and by using a sterile/apyrogenic high-speed diamond bur. The first stage was performed to promote a major removal of contaminants, including carious lesion and restoration. In the second stage, before entering the pulp chamber, the access cavity was disinfected according to the protocol described previously. Sterility of the internal surface of the access cavity was checked as previously described, and all procedures were performed aseptically. An endotoxin sample was taken by introducing sterile/apyrogenic paper points (size #15; Dentsply Maillefer, Ballaigues, Switzerland) into the full length of the canal determined radiographically and retained in position for 60 seconds for sampling. Immediately after, the sample was placed in pyrogen-free glass and immediately suspended in 1 mL limulus amebocyte lysate water and frozen at −80°C for further dosage of endotoxins using a kinetic chromogenic limulus amebocyte lysate assay (Lonza, Walkersville, MD).

After endotoxin sampling, the root canal length was determined from the preoperative radiograph and confirmed using an apex locator (RomiApex A-15; Romidan Dental Solution, Kiryat-Ono, Israel). The root canals were prepared by using Mtwo files (VDW, Munich, Germany) according to the manufacturer's instructions. The files were adapted to an electric motor (VDW), and all instruments were used within the working length in a gentle in-and-out motion. The instrumentation sequence was as follows: 0.04 taper size #10 instrument, 0.05 taper size #15 instrument, 0.06 taper size #20 instrument, 0.06 taper size #25 instrument, 0.05 taper size #30 instrument, 0.04 taper size #35 instrument, 0.04 taper size #40 instrument, and 0.07 taper size #25 instrument. The use of each instrument was followed by irrigation with disposable syringes and 30-G NaviTip needles (Ultradent, South Jordan, UT) using 5 mL 2.5% NaOCl solution. Before the second sampling after instrumentation, NaOCl was inactivated with 5 mL sterile 0.5% sodium thiosulfate during a 1-minute period, which was then removed with 5 mL sterile/apyrogenic water. Next, the second bacterial sampling was performed as previously described.

After root canal instrumentation, the samples were collected from the interstitial fluid in the apical periodontitis according to Martinho et al (31). Three sterile paper points were introduced into the root canal through the root apex (2 mm) and kept there for 1 minute for sampling inflammatory contents from the interstitial fluid in the apical tissues. After withdrawal, the paper points were cut 4 mm from the tip, dropped into a 1.5-mL sterile plastic tube, and immediately stored at −80°C for further analysis of the cytokines.

Quantification of MMPs, TIMPs, and MMP/TIMP Complexes

Quantification of Inflammatory Cytokines (Enzyme-linked Immunosorbent Assay). The amount of MMPs (MMP-1 [Cat #DY901], -2 [Cat#DY902], and -9 [Cat #DY911]), TIMPs (TIMP-1 [Cat# DY970] and -2 [Cat#DY971]), and MMP/TIMP complexes (MMP-1/TIMP-1 [Cat# DY1550], MMP-1/TIMP-2 [Cat #DY1553], MMP-2/TIMP-1 [Cat #DY1496], MMP-2/TIMP-2 [Cat #DY1497], MMP-9/TIMP-1 [Cat #DY1449], and MMP-9/TIMP-2 [Cat#DY1453]) sampled from the interstitial fluid in the apical tissues were measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Inc, Minneapolis, MN). Next, standard and sample solutions were added to the ELISA well plate, which had been precoated with specific monoclonal capture antibody for MMP-1, MMP-2, MMP-3, TIMP-1, TIMP-2, MMP-1/TIMP-1, MMP-1/TIMP-2, MMP-2/TIMP-1, MMP-2/TIMP-2, MMP-9/TIMP-1, and MMP-9/TIMP-2. After it was gently shaken for 3 hours at room temperature, the polyclonal anti–MMP-1, –MMP-2, –MMP-3, –TIMP-1, –TIMP-2, –MMP-1/TIMP-1,