Macrophage Cell Activation with Acute Apical Abscess Contents Determined by Interleukin-1 Beta and Tumor Necrosis Factor Alpha Production

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Abstract

Introduction: This clinical study has investigated the antigenic activity of bacterial contents from exudates of acute apical abscesses (AAAs) and their paired root canal contents regarding the stimulation capacity by levels of interleukin (IL)-1 beta and tumor necrosis factor alpha (TNF-α) throughout the root canal treatment against macrophage cells. **Methods:** Paired samples of infected root canals and exudates of AAAs were collected from 10 subjects. Endodontic contents were sampled before (root canal sample [RCS] 1) and after chemomechanical preparation (RCS2) and after 30 days of intracanal medication with calcium hydroxide + chlorhexidine gel ($Ca[OH]_2$ + CHX gel) (RCS3). Polymerase chain reaction (16S rDNA) was used for detection of the target bacteria, whereas limulus amebocyte lysate was used to measure endotoxin levels. Raw 264.7 macrophages were stimulated with AAA exudates from endodontic contents sampled in different moments of root canal treatment. Enzyme-linked immunosorbent assays were used to measure the levels of TNF-α and IL-1 beta. **Results:** *Parvimonas micra*, *Porphyromonas endodontalis*, *Dialister pneumosintes*, and *Prevotella nigrescens* were the most frequently detected species. Higher levels of endotoxins were found in samples from periapical exudates at RCS1 ($P < .005$). In fact, samples collected from periapical exudates showed a higher stimulation capacity at RCS1 ($P < .05$). A positive correlation was found between endotoxins from exudates with IL-1 beta ($r = 0.97$) and TNF-α ($r = 0.88$) production ($P < .01$). The significant reduction of endotoxins and bacterial species achieved by chemomechanical procedures (RCS2) resulted in a lower capacity of root canal contents to stimulate the cells compared with that at RCS1 ($P < .05$). The use of $Ca(OH)_2$ + CHX gel as an intracanal medication (RCS3) improved the removal of endotoxins and bacteria from infected root canals ($P < .05$) whose contents induced a lower stimulation capacity against macrophages cells at RCS1, RCS2, and RCS3 ($P < .05$). **Conclusions:** AAA exudates showed higher levels of endotoxins and showed a greater capacity of macrophage stimulation than the paired root canal samples. Moreover, the use of intracanal medication improved the removal of bacteria and endotoxins from infected root canals, which may have resulted in the reduction of the inflammatory potential of the root canal content. *(J Endod 2014;40:1752–1757)*

Key Words

Endodontics, endotoxins, instrumentation, root canal and abscess

Acute apical abscesses (AAAs) are mainly caused by bacteria from infected root canals that invade the surrounding tissues, establishing an extraradicular infection and producing a purulent inflammation (1). In the clinical scenario, the disease is characterized by spontaneous pain and/or swelling, which indicates diffusion through the facial tissues, spreading to the sinuses and other facial spaces of the head and neck (2, 3).

The microbiota involved in AAAs seems to present multifaceted niches composed by several bacterial populations (1, 2, 4, 5) with species found in abscesses different from those observed in paired root canals of the same subjects (4). The search for a single or a small group of species responsible for this condition has been ineffective because culture and molecular identification showed a multispecies community involved in AAAs (1, 2, 4). Particularly, gram-negative bacteria play an important role in the inflammatory response mediated by the outer layers of bacterial cell walls, also known as endotoxins or lipopolysaccharides (LPSs) (6).

Endotoxins are potent activators of the immune response, establishing a relevant network in the inflammatory reaction from primary endodontic disease, with posterior development of clinical symptoms and features (7). This complex network seems to be mediated by Toll-like receptor 4 (8) by stimulating the production of proinflammatory cytokines, such as interleukin (IL)-1 beta and tumor necrosis factor alpha (TNF-α) (9). Many previous studies focused on the ability of different root canal procedures in reducing bacterial load and endotoxins from primary endodontic disease

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(10, 11). However, no study has investigated the residual stimulation capacity of bacterial endodontic contents throughout the root canal treatment (2, 12–15).

Bacterial contents from infected root canals are intimately related to the severity of periapical inflammatory disorder, presenting implications on the clinical manifestation of the disease. Therefore, we have hypothesized in the present study that reducing the number of bacterial species and endotoxin levels from infected root canals throughout the root canal treatment would result in a reduction of their stimulation capacity against macrophage cells depending on the release of IL-1 beta and TNF-α.

Therefore, this clinical study investigated the antigenic activity of bacterial contents from exudates of AAAs and their paired root canal contents regarding the stimulation capacity by levels of IL-1 beta and TNF-α throughout the root canal treatment against macrophages cells.

Materials and Methods

Patient Selection

This protocol was approved by the Human Research Ethical Committee of the Piracicaba Dental School, State University of Campinas, UNICAMP, Piracicaba, São Paulo, Brazil. All patients signed an informed consent form before participating in the study. Ten patients complaining of dental pain and soft tissue swelling who were diagnosed with pulp necrosis and AAAs were included. Periapical intraoral radiographs (Insight Dental Films; Eastman Kodak Company, São Paulo, Brazil) were obtained from each tooth using the parallel technique. The radiographs were analyzed for apical periodontitis (bone destruction) present in the apical area from the involved teeth. It is important to emphasize that all teeth presented a radiolucent area at the apex. In addition, the apical condition was determined through the observation of clinical signs, such as tenderness to percussion, pain on palpation, and swelling. Patients received no prior endodontic treatment or antibiotic treatment within the preceding 3 months. Teeth with a periodontal probing depth greater than 4 mm and subjects with systemic diseases were not included in the study.

Root Canal Sampling. All materials, including paper points, burs, and saline solution, were sterilized by gamma radiation with cobalt 60 (3). Teeth were isolated with a rubber dam, disinfected by using 30% hydrogen peroxide and 2.5% sodium hypochlorite, and inactivated with 5% sodium thiosulfate. Field sterility was checked for the absence of bacteria and endotoxins by taking a sterile/apyrogenic swab sample from the crown surface. The bacterial sampling was streaked onto blood agar plates, which were then incubated aerobically and anaerobically, and endotoxin sampling was analyzed by the limulus amebocyte lysate (LAL) technique.

Under anesthesia (2% lidocaine with 1:100,000 epinephrine), a 2-stage access cavity preparation was performed. The access cavity was made without water spray but under manual irrigation with saline solution. This first stage was performed to remove caries. In the second stage, before entering the pulp chamber, the cavity was disinfected as described previously and tested for the absence of bacteria and endotoxins. A new bur was used to achieve the canal under irrigation with saline solution.

For each tooth, only 1 root canal was sampled. If the tooth was multirooted, the canal with periradicular radiolucency was chosen to confine the microbial evaluation to a single environmental site (4). Before the first root canal sample (RCS1), a K-file size #15 (Dentsply Maillefer, Ballaigues, Switzerland) was used in order to confirm the working length previously estimated by radiographs with an apex locator (Novapex; Forum Technologies, Rishon le-Zion, Israel). RCS1 was taken by introducing paper points (Dentsply Maillefer) into the full length of the root canal, which was determined by radiographs and an apex locator (Novapex), and retaining them in position for 60 seconds. Next, the paper points were frozen at −80°C for posterior analysis.

Whenever the paper point was unable to reach the full-length extension of the root canal, teeth were excluded from the study. Moreover, teeth presenting differences between the root canal length determined by preoperative radiography and the apex locator were also excluded.

Root canals were then prepared with Mtwo instruments (VDW, Munich, Germany) in constant rotation at a speed of 300 rpm. Mtwo instruments (10/.04, 15/.05, 20/.06, 25/.06, 30/.05, 35/.04, and 40/.04) were used until reaching the full length of the root canal in a single-length technique, with gentle in-and-out movements while gradually advancing apically (16). Before the use of each instrument, root canals were irrigated with a syringe (27-G needle) containing 1 mL 2% chlorhexidine (CHX) (Endogel; Itapetininga, São Paulo, Brazil); subsequently, 5 mL sterile saline solution was used to wash the canal. CHX activity was inactivated with 5 mL of a solution containing 5% Tween 80 (Drogal; Piracicaba, SP, Brazil) and 0.07% (w/v) lecithin for 60 seconds, which was then removed with 5 mL saline solution. The second sample (RCS2) was immediately taken, as previously described.

Next, the canals were dried with paper points and filled with $Ca(OH)_2$ + 2% CHX gel paste (17), which was plugged into the canal by using Lentulo files (Malleifer Dentsply). Before temporary restoration, sterile cotton was placed in the chamber, and temporary restoration was performed by the "sandwich technique" using 2 layers of Cavit (ESPE, Seefeld, Germany) and light-cured resin (Z-250; 3M Dental Products, St Paul, MN).

After 30 days, the canal was aseptically accessed, and the medication removed with 5 mL saline solution with the assistance of a master apical file. After reinstrumentation, the activity of the CHX present in the root canal medication was inactivated with 5 mL of a solution containing 5% Tween 80 and 0.07% (w/v) lecithin during a 1-minute period, which was then removed with 5 mL saline solution. The $Ca(OH)_2$ activity was neutralized with 0.5% citric acid during a 1-minute period, which was then removed with 5 mL saline solution. Next, the third sample (RCS3) was immediately taken.

Root canals were soaked with 17% EDTA for 3 minutes, which was renewed every minute using 5 mL as the total volume. This solution was activated by pumping a well-fitting gutta-percha master cone previously calibrated at the final apical diameter of the file with short vertical strokes.

After each sample collection (RCS1–RCS3), the procedure was repeated with 5 sterile paper points. The paper points were pooled into a tube containing 1 mL Viability Medium Goteborg Agar III transport medium and immediately processed for DNA extraction to detect target bacteria using the molecular method (16S ribosomal DNA).

AAA Sampling. AAA samples were taken as described elsewhere (3, 4). The oral mucosa was first disinfected with 2% CHX gel, which was then neutralized with a solution containing 5% Tween 80 and 0.07% (w/v) lecithin during a 1-minute period. Next, a gauze piece soaked in saline solution was used to remove traces of these substances. Afterward, field sterility was checked for the absence of bacteria and endotoxins by taking a sterile/apyrogenic swab sample from the target oral mucosa area. The bacterial sampling was streaked onto blood agar plates, which were then incubated aerobically and anaerobically; and the endotoxin sampling was analyzed using the LAL technique.

AAA samples from intact swollen mucosa were taken by aspiration before surgical drainage, with 100 μL being immediately placed into a glass tube and frozen at −80°C for further analysis. The procedure