Quantification of Endotoxins in Infected Root Canals and Acute Apical Abscess Exudates: Monitoring the Effectiveness of Root Canal Procedures in the Reduction of Endotoxins

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Abstract

Introduction: This clinical study was conducted to measure the endotoxin levels in infected root canals (RCs) and exudates related to acute apical abscesses (AAAs). In addition, the effectiveness of RC procedures in reducing the endotoxin levels in RCs was monitored. **Methods:** Paired samples of infected RCs and exudates from AAAs were collected from 10 subjects by using paper points. RCs samples were collected before (RCS1) and after chemomechanical preparation (CMP) (RCS2), after 17% EDTA (RCS3), and after 30 days of intracanal medication ($Ca[OH]_2$ + chlorhexidine) (RCS4). A turbidimetric kinetic limulus amebocyte lysate assay was used for the measurement of endotoxins. **Results:** Endotoxins were detected in 100% of the baseline samples of AAAs and RCs (RCS1) with median values of 175 EU/mL and 41.5 EU/mL, respectively ($P < .05$). After CMP (RCS2), endotoxins were reduced to a median value of 0.54 EU/mL ($P < .05$). Subsequent irrigation with EDTA (RCS3) failed to present a significant effectiveness in reducing the endotoxin levels (median= 0.37 EU/mL) ($P = .07$). However, intracanal medication for 30 days (RCS4) reduced endotoxins to median values of 0.03 EU/mL ($P < .01$). **Conclusions:** The present study revealed a strong association between the high levels of endotoxins found in AAAs and RCs collected from the same tooth. Moreover, the effectiveness of CMP in reducing the endotoxin levels from RCs in acute endodontic infection was improved by the use of RC medication. *(J Endod 2014;40:177–181)*

Key Words

Abscess, chemomechanical preparation, endodontics, endotoxins, intracanal medicament

Apical periodontitis is the result of an inflammatory response to bacteria and their byproduct that invades the periapical tissues via the apical foramen (1–3). The disease presents in different ways, including the development of a form of an acute abscess (1, 2).

Acute apical abscesses (AAAs) present ecologically multifaceted niches that harbor a complex microbial community formed by diverse bacterial populations (1–4) that are different from the ones present in the root canals (RCs) of the same subject (3). Culture and advanced molecular microbiology have shown a multispecies community in AAAs conspicuously dominated by anaerobic bacteria, with a predominance of gram-negative bacterial species of the genera *Fusobacterium, Prevotella*, *Porphyromonas*, *Dialister*, and *Treponema* (1–4).

All these gram-negative bacterial species express virulence factors that play an important role in apical tissue aggression and lesion development (5–10). One of these virulence factors are lipopolysaccharides (LPSs), also known as endotoxins (11–13). Even though these endotoxins are anchored to the outer cell membrane, they can be released during multiplication or bacterial death, thus continuously stimulating the surrounding tissues (11).

LPSs are potent activators of immune cells via mainly the toll-like receptor 4, leading to the release of different proinflammatory cytokines (7, 8, 14). These cytokines establish an important network in the inflammatory reaction (14), which is involved in the development of symptoms and clinical features, such as apical bone destruction and pain (7–9, 14, 15). Therefore, to contribute to the remission of signs and symptoms, clinical features, and bone destruction, the treatment of AAAs should not only aim to kill viable bacteria but also to eliminate/remove endotoxins (5, 16).

Most previous clinical studies focused on the investigation of endotoxins in chronic endodontic infection with apical periodontitis, evaluating different clinical protocols in the removal/elimination of endotoxins (5, 6, 17–20). There is a lack of studies

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investigating the levels of endotoxins in RCs of teeth with AAAs after different clinical steps in RC therapy and endotoxin levels in paired samples of RCs and drained abscesses from the same patient (13, 21). Therefore, this clinical study was conducted to measure the levels of endotoxins present in infected RCs and exudates related to AAAs and to monitor the effect of RC procedures on the endotoxin levels in RCs.

Materials and Methods

Patient Selection

The present study was approved by the Human Research Ethical Committee of the Piracicaba Dental School (State University of Campinas–UNICAMP, Piracicaba, São Paulo, Brazil), and informed consent was obtained from all subjects. Ten patients who attended the local emergency service because of dental pain and soft tissue swelling diagnosed with pulp necrosis and AAAs were included in this study. In addition, teeth presented tenderness to palpation and/or percussion and a radiolucent area around the apex. The pulp status was assessed through thermal vitality tests, and the apical condition was determined through the observation of clinical signs, such as tenderness to percussion and pain on palpation. The selected teeth had received no prior endodontic treatment. Subjects who received antibiotic treatment within the preceding 3 months, teeth with periodontal probing depth >4 mm, and subjects with systemic diseases were not included in the study.

Endotoxin Sampling

RC Sampling. All materials used in this study, including sterile burs, tweezers, paper points, saline, and glass tubes, were also sterilized by gamma radiation with cobalt 60 (20 KGy for 6 hours) (22) to become endotoxin free (or apyrogenic). Teeth were isolated from the oral cavity with a rubber dam, and disinfection of their external surfaces and surrounding areas was performed by using 30% hydrogen peroxide followed by 2.5% sodium hypochlorite (NaOCl). Subsequently, 5% sodium thiosulfate was used to inactivate the disinfectant agents. 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 aerobically and anaerobically.

A 2-stage access cavity preparation was performed. The first stage involved the removal of caries and the associated microorganisms and endotoxins without exposure of the pulp chamber. The access cavity was made without water spray but under manual irrigation with saline and by using a high-speed diamond bur.

In the second stage, before entering the pulp chamber, the access cavity was disinfected according to the decontamination protocol described previously. Sterility was also checked by taking swab samples from the cavity surface and streaking them onto blood agar plates, with subsequent incubation at 37°C under both aerobic and anaerobic conditions.

The access cavity was likewise tested for the presence or absence of endotoxins before and after entering the pulp chamber, which was determined by the limulus amebocyte lysate (LAL) assay as described previously. A new bur was used under irrigation with saline to access the canal.

The sampling included only 1 RC for each tooth in order to confine the microbial evaluation to a single environmental site (3). Particularly, in multirooted teeth, the canal selected was firstly the one related with the periapical radiolucency, otherwise, the largest canal.

The first endotoxin sample (RCS1) was taken by introducing the paper points (Dentsply Maillefer, Ballaigues, Switzerland) into the full length of the canal (determined by radiographs and apex locator) and retaining them in position for 60 seconds. Next, the paper points were immediately placed on a glass tube and frozen at 80°C for the LAL assay.

Whenever it was not possible to place the paper point into the full-length extension of the RC, teeth were excluded from the present study. Moreover, those teeth that had differences between the length of the canal determined by a preoperative radiograph and the apex locator were also excluded from this study.

After the first sampling, the RC length was determined from the preoperative radiograph and confirmed by the apex locator (Novapex, Forum Technologies, Rishon le-Zion, Israel). The RCs 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 up to the full length of the canal in a single-length technique, with a gentle in-and-out movement while gradually advancing apically (16). Before the use of each instrument, RCs were irrigated with a syringe (27-G needle) containing 1 mL 2% chlorhexidine (CHX) (Endogel, Itapetininga, SP, Brazil); subsequently, 5 mL saline solution was used to wash the canal.

After instrumentation, CHX activity was inactivated with 5 mL of a solution containing 5% Tween 80 (Sigma-Aldrich, St. Louis, MO) and 0.07% (w/v) lecithin during a 1-minute period, which was then removed with 5 mL saline solution. The second endotoxin sample (RCS2) was taken as previously described just after the irrigation with saline solution.

The canals were then irrigated with a continuous rinse with 5 mL 17% EDTA solution for 3 minutes followed by a final rinse with 5 mL saline solution. After this, the third endotoxin sample (RCS3) was taken.

Afterwards, the canals were dried with paper points and filled with $Ca(OH)_2$ + 2% CHX gel paste (23). Paste was plugged into the canal by using Lentulo files (Dentsply Maillefer) and the blunt end of a paper point. The access cavities were properly filled with 2 layers of Cavit (ESPE, Seefeld, Germany) and light-cured resin (Z-250; 3M Dental Products, St Paul, MN).

After 30 days of intracanal medication, the canal was aseptically accessed under rubber dam isolation by using the protocol for disinfection described previously. The medication was removed with 5 mL saline solution and by carefully filing the canal with a master apical file. After reinstrumentation, the activity of the CHX present in the RC 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. Then, the fourth and final endotoxin sample (RCS4) was taken as described earlier just after the irrigation with saline solution.

After suctioning away the intracanal surplus of sterile/apyrogenic saline solution, the RCs were soaked with 17% EDTA during a 3 minute-period, which was renewed every minute, using 5 mL as a total volume. This solution was activated by pumping a well-fitting gutta-percha master cone previously calibrated at the final apical file diameter with short vertical strokes.

Afterwards, a final rinse with 5 mL saline solution was performed to remove traces of EDTA. The canals were subsequently dried with paper points and filled using vertical and lateral compaction of gutta-percha cones (Konne, Belo Horizonte, MG, Brazil) with Endométhasone sealer (Septodont, Saint-Maur-des-Fossés, France). The access cavities were restored with a 2-mm layer of Coltosol (Coltène Whaledent, Cuyahoga Falls, OH) and Filtek Z250 (3M Dental Products, St Paul, MN).

AAA Samplings. AAAs samples were taken as described previously by Rôças et al (24) and Montagner et al (3), The oral mucosa was first disinfected with 2% CHX gel, which was neutralized with a solution containing 5% Tween 80 and 0.07% (w/v) lecithin for 1 minute.