Quantification of Lipoteichoic Acid Contents and Cultivable Bacteria at the Different Phases of the Endodontic Retreatment

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Abstract

Introduction: The infectious content of root canals, including bacteria and lipoteichoic acid (LTA), cause injuries to the periapical tissues. The purpose of this clinical study was to quantify the levels of both LTA and cultivable bacteria at the different phases of endodontic retreatment (ER) of teeth with post-treatment apical periodontitis. It also aimed to investigate the presence of gram-positive microorganisms before and after chemomechanical preparation (CMP) and intracanal medication (ICM). **Methods:** Twenty infected root canals of single-rooted teeth were randomly assigned into 2 groups according to the chemical substance used for CMP (n = 10 per group): chlorhexidine (CHX) group, 2% CHX gel, and the sodium hypochlorite (NaOCl) group, 6% NaOCl. Root canal samples were taken using paper points before (S1) and after CMP (S2) and after 30 days of ICM with calcium hydroxide + 2% CHX gel (S3). Microorganisms were identified by the culture technique using biochemical tests. Cultivable bacteria were determined by counting the colony-forming unit. LTA levels were measured using the enzyme-linked immunosorbent assay (pg/mL). **Results:** A total of 70 gram-positive species, out of 102 species isolated, were found in the root canals (54 in S1, 4 in S2, and 12 in S3). *Enterococcus faecalis* was the most frequent isolated taxon in all phases of the ER. LTA (574.0 ± 94.7) and cultivable bacteria (101.2 ± 79.2) were present in all S1 samples. CMP decreased the overall levels of cultivable bacteria by 99.4% and LTA by 24.8% ($P < .05$), whereas the total overall reduction level of ICM on viable bacteria was 99.5% and on LTA it was 38.6% ($P < .05$). CMP with 2% CHX gel (CHX group, 99.3%) was more effective ($P < .05$) than 6% NaOCl (NaOCl group, 92.1%) on bacterial reduction. Likewise, ICM showed a 100% reduction in the CHX group and 98.5% in the NaOCl group. Regarding the reduction of LTA, CMP with 2% CHX gel (CHX group, 26.9%) was more effective ($P < .05$) than 6% NaOCl (NaOCl group, 22.6%). In addition, ICM showed a 43.2% reduction in the CHX group and 36.2% in the NaOCl group ($P > .05$). **Conclusions:** The reduction rates of bacteria were higher than the LTA. Moreover, gram-positive microorganisms were present in all phases of the endodontic retreatment. *(J Endod 2016;42:552–556)*

Key Words

Bacteria, chlorhexidine, *Enterococcus faecalis*, sodium hypochlorite, virulence factor

The role of microorganisms in the pathogenesis of apical periodontitis has been nicely elucidated by the literature (1–4) because they can perpetuate an infection after root canal filling or induce new inflammation in the periapical region (3, 5, 6).

The microbiota of teeth with failure of endodontic treatment is predominantly composed of facultative anaerobic microorganisms such as *Actinomyces*, *Candida*, and *Enterococcus* species (7, 8). *Enterococcus faecalis*, a facultative gram-positive bacterium, is one of the most detected microorganisms in retreatment cases, using culture-dependent or independent techniques, being able to endure severe conditions of survival in the root canal with a large variation of pH, temperature, and O_2 tension (9–12). When exposed to environmental stress, some strains can adopt a viable existing state and resuscitate when normal conditions are re-established (13).

Even though *E. faecalis* was found in canals with or without periapical lesions (14), its role in endodontic failure is still controversial. Nevertheless, *E. faecalis* was found in the root canals in higher numbers, before and after mechanical preparation, using next-generation sequencing (15), confirming their resistance to the endodontic procedures.

Even after bacterial death, some components of the gram-positive bacteria cell wall (eg, lipoteichoic acid [LTA]) persist in the root canal for long periods of time, which can cause chronic inflammation (6, 11, 16–18).

The antigenic complexity of the root canal content is influenced by the diversity and the number of microbial species, including synergistic and antagonistic relationships and the presence of bacterial virulence factors (eg, LTA), modulating the toxicity of this infectious content (19, 20).

LTA is an important virulence factor of gram-positive bacteria (9, 21–24), which is released during bacterial multiplication, mainly after bacteriolysis by lysozyme, bactericidal cationic peptides, phospholipase A, cathepsins, or beta-lactam antibiotics (21). It has pathogenic properties similar to the lipopolysaccharides (LPSs) of

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gram-negative bacteria (3, 18, 21, 23–25), resulting in well-known injuries to dental pulp and periapical tissues. However, LTA differs from LPSs in terms of structure and gene transcription (24).

LTA can begin a series of reactions by binding to specific receptors (CD14 and toll-like 2 receptors) or by nonspecific events (phospholipid membrane; complement system activation; release of proinflammatory cytokines such as tumor necrosis factor alpha, interleukin [IL]-1β, IL-6 IL-8, and prostaglandin E_2 (PGE_2); angiogenesis regulation; release of hydrolases, proteases, prostaglandins, and reactive oxygen species from neutrophils and macrophages; and regulation, recruitment, and activation of neutrophils) (3, 17, 18, 21–26).

Thus, the present study aimed to investigate the levels of LTA and the cultivable gram-positive microorganisms and to evaluate the effect of chemomechanical preparation (CMP) and intracanal medication (ICM) on the reduction of LTA and bacteria of teeth with post-treatment apical periodontitis.

Materials and Methods

Patient Selection

Twenty patients were selected from those who attended the Piracicaba Dental School, State University of Campinas–UNICAMP, Piracicaba, São Paulo, Brazil, with a need for nonsurgical endodontic retreatment.

The Human Volunteers Research and Ethics Committee of the Piracicaba Dental School approved a protocol (# 018/2014) describing the specimen collection for this investigation, and all patients signed an informed consent for their participation in this research. The age of the patients ranged from 30–60 years. All selected teeth (n = 20) had been previously single root filled and showed radiographic evidence of apical periodontitis.

Failure of root canal treatment was determined based on clinical and radiographic examinations. The presence of persistent periapical radiolucent lesions; voids in or around the root canal filling; persistent symptoms such as pain of palpation, discomfort to percussion, and persistent sinus tract were considered reasons for retreatment (7).

Exclusion criteria were as follows:

- Subjects who had received antibiotic treatment within the preceding 3 months
- Subjects who reported systemic disease starting with American Society of Anesthesiologists grade 3
- Subjects who had teeth that could not be isolated with a rubber dam, teeth with absence of coronary sealing, or teeth with periodontal pockets deeper than 3 mm

Endodontic Sample Collection and Clinical Procedures

The teeth were isolated with a rubber dam. The crown and surrounding structures were disinfected with 30% hydrogen peroxide (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 (8, 27). Disinfection 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.

The sampling procedures were performed according to Endo et al (8) and Martinho and Gomes (27). Under anesthesia (2% lidocaine with 1:100,000 epinephrine), a 2-stage access preparation was performed. The access cavity was made without the use of water spray but under manual irrigation with sterile saline and by using sterile high-speed diamond bur. This first stage was performed to promote a major removal of contaminants. In the second stage, before entering the pulp chamber, the access cavity was disinfected according to the decontamination protocol described previously. Disinfection of the internal surface of the access cavity was checked as previously described, and all procedures were performed aseptically. Root filling materials were removed by using Reciproc R25 files (VDW, Munich, Germany) in the working length obtained by preoperative radiography and used according to the manufacturer's instructions in a crown-down technique with no chemical solvent.

Before the first sample (S1) of the root canal, a K-file #20 (Dentsply Maillefer, Ballaigues, Switzerland) was used to confirm the working length (previously estimated by radiographs) with an apex locator (Novapex; Forum Technologies, Rishon le-Zion, Israel). A sterile paper point (Dentsply Maillefer) was then introduced into the full length of the canal and retained in position for 60 seconds for LTA sampling. Next, this paper point was placed in a sterile tube for the enzyme-linked immunosorbent assay (ELISA). Three paper points were pooled in a sterile tube containing 1 mL Viability Medium Göteborg Agar (VMGA III) transport medium (28) for microbial sampling. The samples were transported within 15 minutes to an anaerobic workstation (Don Whitley Scientific, Bradford, UK) for bacterial culture analysis. The LTA samples were frozen at −80°C for further analysis.

Root canals were then prepared by using Reciproc R40 files (VDW) according to the manufacturer's instructions in a reciprocating working motion generated by the motor. The instrument was used in an in-an-out pecking motion of about 3 mm in amplitude with apical pressure. After 3 pecking motions, the instrument was removed from the canal and cleaned. Next, a K-file #20 was taken to the working length to check whether the canal was patent. These procedures were repeated until the Reciproc instrument reached the working length (0 point displayed on the apex locator).

Retreatment was deemed complete when the Reciproc R40 file reached the working length, with no filling material covering the instrument and the canal walls smooth and free of visible debris. Furthermore, close inspection under high magnification with a dental operating microscope (DF Vasconcelos SA, São Paulo, SP, Brazil) showed complete removal of gutta-percha.

The 20 infected root canals of single-rooted teeth with post-treatment apical periodontitis were divided randomly into 2 groups according to the chemical substances used: the chlorhexidine (CHX) group (n = 10), 2% CHX gel, and the NaOCl group (n = 10), 6% NaOCl.

The EndoVac System (Discus Dental, Culver, CA) was used to irrigate both groups using saline in the CHX group and NaOCl in the NaOCl group. In the CHX group, during instrumentation, the root canals were filled with 1 mL 2% CHX gel (Endogel) using a syringe (27-G needle) before the use of each instrument and immediately rinsed afterward with 5 mL saline solution using the EndoVac System. At the end of the instrumentation, CHX was inactivated with 5 mL 5% Tween 80 (Drogal, Piracicaba, SP, Brazil) and 0.07% (w/v) lecithin solution for a 1-minute period, which was removed with 5 mL saline solution.

In the NaOCl group, during instrumentation, the root canals were filled with 1 mL 6% NaOCl using a syringe (27-G needle) before the use of each instrument and immediately rinsed afterward with 5 mL 6% NaOCl using the EndoVac System. At the end of the instrumentation, NaOCl was inactivated with 5 mL of a solution of 5% sodium thiosulfate (Drogal) for 60 seconds, which was also removed with 5 mL saline solution.

Before the second sampling procedure (S2), a rinse with 5 mL 17% EDTA was applied continuously for 3 minutes under stirring with ultrasound (Advanced SE; Microdont, São Paulo, SP, Brazil) with tip ET40 (Satelec/Acteon, Mount Laurel, NJ) for 60 seconds alternately followed by a final rinse with 5 mL sterile saline solution. Next, second LTA and microbiological samples were taken (S2) as previously described.

The canal was dried with paper points. A calcium hydroxide paste prepared with 2% CHX gel (1:1) was placed over the entire length of the