Effects of Chemomechanical Preparation With 2.5% Sodium Hypochlorite and Intracanal Medication With Calcium Hydroxide on Cultivable Bacteria in Infected Root Canals

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Abstract

This clinical study was conducted to assess the bacterial reduction after chemomechanical preparation with 2.5% NaOCl as an irrigant and the additive antibacterial effect of intracanal dressing with calcium hydroxide. According to stringent inclusion criteria, 11 teeth with primary intraradicular infections and chronic apical periodontitis were selected and monitored in the study. Bacterial samples were taken at the baseline (before treatment) (S1), after chemomechanical preparation with 2.5% NaOCl as an irrigant (S2), and after a 7-day dressing with a calcium hydroxide paste in glycerin (S3). Cultivable bacteria recovered from infected root canals at the 3 stages were counted and identified by means of 16S rRNA gene sequencing analysis. At S1, all canals were positive for bacteria, with the mean number of 2.8 taxa per canal (range, 1–6). At S2, 5 cases (45.5%) still harbored cultivable bacteria, with 1 or 2 species per canal. At S3, bacteria were cultured from 2 cases (18.2%), with 1 species per positive case. There was no indication that any specific bacterial taxon was more resistant to treatment. A significant reduction in bacterial counts was observed between S1 and S2, and S1 and S3. However, no statistically significant difference was observed for comparisons involving S2 and S3 samples with regard to the number of cases yielding negative cultures ($P = .18$) or quantitative bacterial reduction ($P = .19$). It was concluded that the whole antibacterial protocol used in this study significantly reduced the number of bacteria in the canal and rendered most canals free of cultivable bacteria. *(J Endod 2007;33:800–805)*

Key Words

16S rRNA gene sequencing, antimicrobial treatment, apical periodontitis, endodontic microbiology

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Primary intraradicular infections are largely nonspecific and predominantly anaerobic, with no single species being considered as the main pathogen. These infections display a large interindividual variability, ie, each individual exhibits a unique endodontic microbiota in terms of composition and species dominance (1, 2). Consequently, the endodontic treatment should rely on a broad-spectrum antimicrobial strategy to deal with such infections.

Because of the privileged anatomic localization, bacteria entrenched in the root canal system are beyond the reaches of the host defenses. Therefore, endodontic infections can only be treated by means of professional intervention with both chemical and mechanical procedures. The main steps of endodontic treatment involved with control of the infection are represented by the chemomechanical preparation and the interappointment medication. In this regard, the chemomechanical preparation is of paramount importance for root canal disinfection, because instruments and irrigants act primarily on the main canal, which is the most voluminous area of the system and consequently harbors the largest number of bacterial cells. Bacterial elimination from the root canal is carried out by means of the mechanical action of instruments and the flow and backflow of the irrigant solution as well as the antibacterial effects of irrigants. Several irrigants have been proposed over the years, but sodium hypochlorite (NaOCl) remains the most widely used one. However, studies have revealed that instrumentation and irrigation with NaOCl per se do not suffice to predictably render root canals free of cultivable bacteria (3–7). About 40%–60% of the canals still contain cultivable bacteria after chemomechanical preparation with different NaOCl concentrations (3–7). This is because endodontic instruments have a design and rigidity that allow them to act only in the main canal, and NaOCl remains in the canal for a short period of time, which might be insufficient to reach other areas of the root canal system, including irregularities, lateral canals, isthmuses, apical deltas, and dentinal tubules.

The use of an interappointment antibacterial medication has been recommended mostly to eliminate bacteria not affected by the chemomechanical preparation. Calcium hydroxide is arguably the most commonly used intracanal medication, and several clinical studies have reported on its antibacterial activity (3, 5, 7–15). Nevertheless, its effectiveness in significantly increasing the number of culture-negative canals after chemomechanical procedures has been somewhat inconsistent (5, 11, 13).

Most clinical studies investigating the antibacterial effects of intracanal procedures have provided only quantitative data with no species identification. The knowledge of the species that can be resistant to treatment assumes special relevance to the establishment of therapeutic strategies effective in eliminating these species. The few studies that have identified bacteria persisting treatment have made use of phenotypic characterization of the isolates. However, it is well-known that a precise bacterial identification cannot always be achieved with phenotype-based methods. The 16S rRNA gene sequencing method has emerged as a valuble tool for the identification of bacterial isolates, and unlike phenotype-based tests, this molecular approach can provide unambiguous data for identification of isolates that are unreactive in biochemical tests, species with atypical phenotypes, rare isolates, or poorly described bacteria (16). Furthermore, the technique can lead to recognition of novel species and previously uncultivated bacteria (16). Identification of cultivable bacteria on the basis of the 16S rRNA gene sequencing has been recently adopted by many clinical microbiology laboratories to identify rarely

isolated or previously uncharacterized species as well as isolates that are difficult to identify by conventional methods (17).

The purpose of the present study was to assess the effects of chemomechanical preparation with 2.5% NaOCl as an irrigant and intracanal dressing with calcium hydroxide on cultivable bacteria from primary intraradicular infections of teeth with apical periodontitis. Cultivable bacteria recovered from infected root canals at 3 treatment stages were identified by means of 16S rRNA gene sequencing analysis.

Material and Methods

Clinical Material

Patients presenting to the endodontic clinic at the School of Dentistry, Estácio de Sá University, Rio de Janeiro, RJ, Brazil for evaluation and treatment of apical periodontitis were considered for this study. Twelve single-rooted teeth (4 maxillary central incisors, 4 maxillary lateral incisors, 3 mandibular incisors, and 1 mandibular premolar) from 10 patients (8 women and 2 men; aged 24–54 years; mean, 38.7 years) were selected for this study on the basis of stringent criteria. Only teeth with intact pulp chamber walls, necrotic pulps as confirmed by negative response to sensitivity pulp tests, and clinical and radiographic evidence of chronic apical periodontitis lesions were included in this study. The size of the apical periodontitis lesions ranged from 2 × 3 mm to 12 × 15 mm. Teeth from patients who received antibiotic therapy within the previous 3 months, teeth with gross carious lesions, teeth with fractures of the root or crown, teeth that had received previous endodontic treatment, and cases showing periodontal pockets greater than 4 mm deep were excluded from the study. Approval for the study protocol was obtained from the Ethics Committee of the Estácio de Sá University.

Endodontic Treatment and Sampling Procedures

Rubber dam and an aseptic technique were used throughout the endodontic treatment. Before isolation with rubber dam, each tooth had supragingival plaque removed by scaling and cleansing with pumice. Caries and/or coronal restorations were removed with sterile high-speed and low-speed burs. After rubber dam application, dental floss was securely tied around the neck of the tooth. The operative field, including the tooth, clamp, and surroundings, were cleaned with 3% hydrogen peroxide until no further bubbling of the peroxide occurred. All surfaces were then disinfected by vigorous swabbing with a 2.5% NaOCl solution. After completing the access with another sterile bur under sterile saline irrigation, the operative field, including the pulp chamber, was then cleaned and disinfected once again the same way as above. NaOCl was neutralized with 5% sodium thiosulfate, and then sterility control samples were taken from the tooth surface with sterile paper points. For inclusion of the tooth in the study, these control samples had to be uniformly negative.

The first root canal sample (S1) was taken as follows. Three sterile paper points were consecutively placed in the canal to a level approximately 1 mm short of the tooth apex, on the basis of diagnostic radiographs, and used to soak up the fluid in the canal. Each paper point was left in the canal for at least 1 minute. Paper points were then transferred aseptically to tubes containing 500 μL of reduced transport fluid (RTF).

Chemomechanical instrumentation was completed at the same appointment in all cases. The alternated rotation motion (ARM) technique was used to prepare all canals (18). Briefly, the coronal two thirds of the root canals were enlarged with Gates-Glidden (Moyco Union Broach, York, PA) burs. Working length was established 1 mm short of the root apex, and the patency length coincided with the radiographic root edge. Apical preparation was completed to the working length with hand nickel-titanium files (Nitiflex; Dentsply-Maillefer, Ballaigues, Switzerland), always using a back-and-forth ARM. Master apical files ranged from #50 to #55, depending on both root anatomy and initial diameter of the root canal. Apical patency was confirmed with a small file (#15 or #20 NitiFlex) throughout the procedures after each larger file size. Preparation was completed by using step-back of 1-mm increments. NaOCl (2.5%) was used as an irrigant during the preparation. Two milliliters of this solution was used to rinse the canals after each instrument. Irrigant was delivered in the canals by means of a 5-mL disposable syringe with a 23-gauge needle.

Each canal was dried by using sterile paper points and then flushed with 5 mL of 5% sodium thiosulfate to inactivate the NaOCl. Subsequently, the root canal walls were gently filed, and a postinstrumentation sample (S2) was taken from the canal as described above.

To remove the smear layer, 17% ethylenediaminetetraacetic acid was left in the canal for 3 minutes followed by irrigation with 5 mL of 2.5% NaOCl. The canal was dried with paper points and dressed with a mix of calcium hydroxide in glycerin, which was placed in the canals by means of lentulo spiral fillers. The calcium hydroxide slurry was packed with a cotton pellet at the level of canal entrance. A radiograph was taken to ensure proper placement of the calcium hydroxide in the canal. The access cavities were filled with at least 4-mm thickness of a temporary cement (Coltosol; Coltène/Whaledent Inc, Cuyahoga Falls, OH).

The second appointment was scheduled for 1 week thereafter. At this time, the tooth was isolated with a rubber dam, the operative field was disinfected, and the NaOCl was neutralized, as outlined earlier. A sterility control sample of the operating field was obtained. The temporary filling was removed, and the calcium hydroxide paste was rinsed out of the canal by using sterile saline solution and the master apical file. The root canal walls were filed lightly to remove loose calcium hydroxide remnants, and a postmedication sample (S3) was taken from the canals. Subsequently, the canals were filled with gutta-percha and Sealer 26 (Dentsply, Petrópolis, RJ, Brazil) by using cold lateral compaction. The tooth was sealed temporarily with glass ionomer cement, and a permanent restoration was planned. All clinical procedures were conducted by one experienced endodontist (T.G.P.).

Microbiologic Analysis

Samples were transported to the laboratory within 15 minutes for microbiologic processing. Samples in RTF vials were dispersed with a vortex for 30 seconds, and 10-fold serial dilutions to 10^{-3} (for S1 samples) or 10^{-2} (for S2 and S3 samples) were made in prereduced anaerobically sterilized buffered salt solution. Aliquots of 100 μL from the undiluted suspension and the highest dilution were each spread onto Brucella agar plates (BBL Microbiology Systems, Cockeysville, MD) supplemented with 5% defibrinated sheep blood, hemin (5 mg/L), and menadione (1 mg/L), and Mitis-salivarius agar plates (Difco, Detroit, MI). Plates were incubated anaerobically within anaerobic jars (GasPak system; BBL Microbiology Systems) at 37°C for 14 days. After incubation, the total colony-forming units (CFUs) were counted, and actual counts were calculated on the basis of the known dilution factors. One or 2 colonies of each different colony type were isolated, and each one was individually placed in cryovials containing TE buffer (10 mmol/L Tris-HCl, 1 mmol/L ethylenediaminetetraacetic acid, pH 8). Cryovials were then stored at –20°C until further bacterial identification by 16S rRNA gene sequencing.

16S rRNA Gene Identification

Genomic DNA was extracted from each colony by heating the suspension for 10 minutes at 97°C with a thermocycler. The vials were then stored for 5 minutes on ice and centrifuged, and 5-μL aliquots of the supernatant were further used as template in the polymerase chain reaction (PCR) assay.

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