Microbiological Profile Resistant to Different Intracanal Medications in Primary Endodontic Infections

Nadia S. Ferreira, DDS, MSc, PhD, Frederico C. Martinho, DDS, MSc, PhD,* Flavia G.R. Cardoso, DDS, MSc, PhD,* Gustavo G. Nascimento, DDS, MSc,† Cláudio A.T. Carvalho, DDS, MSc, PhD,* and Marcia C. Valera, DDS, MSc, PhD**

Abstract

Introduction: This clinical study aimed to determine the microbiological profile resistant to different intracanal medications in primary endodontic infections by using both microbiological culture and the checkerboard DNA-DNA hybridization technique. **Methods:** Twenty primarily infected root canals were selected and then instrumented before being randomly divided into 2 groups according to the intracanal medications: calcium hydroxide ($Ca[OH]_2$) or $Ca(OH)_2$ + chlorhexidine (CHX). Samples were collected before and after root canal procedures, which consisted in submitting them to microbiological culture and processing them for checkerboard DNA-DNA hybridization. **Results:** No differences were found between the $Ca(OH)_2$ (99.98%) and $Ca(OH)_2$ + CHX groups (99.76%) regarding the median percentage values for the reduction of cultivable bacteria. The most frequently detected species were *Capnocytophaga ochracea* (70%) and *Fusobacterium nucleatum ssp. vincentii* (70%) in the initial samples. After instrumentation, the most frequently detected species were *E. faecium* (60%). After root canal treatments using either $Ca(OH)_2$ or $Ca(OH)_2$ + CHX as intracanal medications, the most frequently detected species were *F. nucleatum ssp. vincentii* (90%) and *Enterococcus faecium* (40%), respectively. Both treatments significantly decreased the number of bacterial species compared with the initial sample. However, this reduction was significantly greater in the $Ca(OH)_2$ + CHX group ($P < .05$). This difference was also observed when evaluating the total bacterial load ($P < .05$). **Conclusions:** The use of $Ca(OH)_2$ associated with CHX as an intracanal medication showed better results by acting on gram-positive and gram-negative microorganisms although such an action to eradicate enterococci should also be sought. *(J Endod 2015;41:824–830)*

Key Words

Bacteria, checkerboard DNA-DNA hybridization, endodontic infection

Apical periodontitis is an infectious disease caused by microorganisms colonizing the root canal system. If bacteria persist after root canal treatment, there is an increased risk of an adverse outcome of the endodontic treatment. Therefore, bacterial presence in the root canal at the time of filling has been shown to be a risk factor for post-treatment apical periodontitis (1).

The microorganisms present in endodontic infections can be significantly reduced with the chemomechanical preparation of root canals (2, 3). However, mechanical instrumentation does not completely eliminate the microbiota present in the root canal. Therefore, the use of an intracanal medication with antimicrobial activity between therapy sessions has been recommended to eliminate possible persistent microorganisms (4–6).

Calcium hydroxide ($Ca[OH]_2$) has been widely used as an intracanal medication because of its antimicrobial capacity and ability to dissolve tissue and induce mineralization (7, 8). Its antimicrobial property is associated with its dissociation into calcium and hydroxyl ions, which produces an alkaline pH, causing a destructive effect on the bacterial membrane (9). Despite the favorable properties of $Ca(OH)_2$, other compounds (eg, chlorhexidine [CHX]) have been associated in order to enhance its antimicrobial spectrum with the aim of targeting bacteria resistant to $Ca(OH)_2$. CHX has an antimicrobial effect, substantivity, and biocompatibility (10–12). However, in clinical practice, controversy exists on whether $Ca(OH)_2$ associated with CHX can improve the removal or elimination of bacteria from infected root canals. The hybridization DNA-DNA checkerboard method can provide information about the bacterial profile in canals with necrotic pulp and also monitor the bacterial profile resistant to treatments, aiding in better use of the available substances. Therefore, this clinical study aimed to determine the microbiological profile resistant to different intracanal medications in primary endodontic infections by using both microbiological culture and the checkerboard DNA-DNA hybridization technique.

Materials and Methods

Patient Selection

Twenty patients referred to the São José dos Campos Dental School (Universidade Estadual Paulista), São José dos Campos, SP, Brazil, for primary endodontic treatment participated in the present study. This work was approved by the local human research

From the *Department of Restorative Dentistry, Universidade Estadual Paulista, São José dos Campos, São Paulo, Brazil; and †Pelotas School of Dentistry, Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil.

Address requests for reprints to Dr Marcia Carneiro Valera, Av Eng Francisco José Longo, 777, 12245-000, São José dos Campos, SP, Brasil. E-mail address: marcia@fosjc.unesp.br

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ethics committee. All patients were volunteers and signed an informed consent form. A detailed dental history was obtained from each patient. Those who had received antibiotic treatment during the past 3 months or who had any general disease were excluded.

All the teeth were single rooted with primary endodontic infection, radiographic evidence of apical periodontitis, and intact pulp chamber walls and showed a presence of 1 root canal. The diagnosis of pulpal necrosis was confirmed by a negative response to the cold test. Teeth that could not be isolated with a rubber dam and with periodontal pockets deeper than 4 mm were excluded. None of the patients reported spontaneous pain. Four cases presented either tenderness to percussion and/or pain on palpation.

Sample Collection

Instruments and all materials used in this study were treated with Co^{60} gamma radiation (20 kGy for 6 hours) for sterilization (EMBRARAD; Empresa Brasileira de Radiação, Cotia, SP, Brazil). Samples were collected under aseptic conditions. The teeth were isolated with a rubber dam. The crown and surrounding structures were disinfected with 30% hydrogen peroxide followed by 5.25% sodium hypochlorite (NaOCl) and subsequently 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 on 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 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.

Root canal samples were taken as follows: 3 sterile paper points were consecutively introduced into the full length of the canal, which was determined radiographically, and retained in position for 60 seconds. Immediately after, the sample was placed in a sterile tube containing 1 mL VMGA III transport medium.

After the first sampling (s1), 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, München, 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 (−1 mm) 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.

Irrigation was performed with disposable syringes and 30-G Navi-Tip needles (Ultradent, South Jordan, UT) using 5 mL 2.5% NaOCl solution between instrumentations. The root canal instrumentation was completed in a single appointment in all cases.

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 saline solution, and then the second sampling (s2) was similarly performed.

At the end of instrumentation, the root canals were flooded with 17% EDTA during a 3-minute period. Next, a final rinse with 5 mL sterile saline solution was performed, and then the root canals were dried by using sterile paper points before being randomly divided into 2 groups of 10 samples each: G1: $Ca[OH]_2$ medication and G2: 2% CHX (Consepsis V; Ultradent Products Inc, South Jordan, UT) + $Ca(OH)_2$ medication.

The root canals were filled with a freshly prepared paste of $Ca(OH)_2$ in a sterile saline solution or 2% CHX for a period of 14 days. The paste was inserted into the canals with the aid of a Lentulo spiral. Care was taken to properly fill the root canal with the $Ca(OH)_2$ paste without any radiographically visible air bubbles. The paste was condensed at the canal orifice level with the aid of a sterile cotton pellet. Next, the access cavities were properly closed with ionomer cement.

After 14 days with intracanal medication, the teeth had their surgical field isolated and disinfected, including removal of the provisional restoration. Next, the root canals were irrigated with 10 mL saline solution, and another collection of bacterial material was performed (s3) in the same manner as described for s1. In the $Ca(OH)_2$ + CHX group, the intracanal medication was removed with 5 mL saline solution; CHX activity was inactivated with 5 mL solution containing 5% Tween 80 and 0.07% lecithin (Biofórmula – Farmácia de Manipulação, São José dos Campos, SP, Brazil), which was then removed with 5 mL saline solution.

Culture Procedure

The transport media containing the root canal samples were thoroughly shaken for 60 seconds (Vortex; Marconi, Piracicaba, São Paulo, Brazil). Serial 10-fold dilutions were made up to 10^{-3}. Fifty microliters of the serial dilutions were plated onto 5% defibrinated sheep blood fastidious anaerobe agar (FAA; Lab M, Bury, UK) by using sterile plastic spreaders to culture nonselective obligate anaerobes and facultative anaerobes. The plates were incubated at 37°C in anaerobic atmosphere for up to 14 days. After this period, colony-forming units (CFUs) were visually quantified for each plate.

Microbiological Assessment: Checkerboard DNA-DNA Hybridization

Three hundred microliters of VMGA containing the root canal samples was transferred to another sterile tube. After this procedure, the tubes were centrifuged at 8000 rpm for 5 minutes. The supernatant was then discarded and the pellet resuspended at 150 μL Tris-EDTA buffer (10 mmol/L tris [hydroxymethyl] aminomethane [Tris]-HCl, 1 mmol/L EDTA, pH = 7.6). Next, 100 μL 0.5 mol/L NaOH was added to each tube, and the samples were frozen at −20°C until they were processed.

Presence, levels, and proportions of 40 bacterial species (Table 1) were determined by the checkerboard DNA-DNA hybridization method described by Socransky et al (13). The DNA probes were prepared by using the DIG DNA Labeling Kit (Roche Diagnostics, Indianapolis, IN) and frozen until the time of use (14). Next, the samples were boiled for 10 minutes, and 800 μL 5 mol/L ammonium acetate was added to promote bacterial lyses and consequent suspension of DNA in solution. A nylon membrane (15 × 15 cm) with a positive charge (Hybond N +; GE Healthcare Limited, Buckinghamshire, UK) was placed in a MiniSlot 30 (Immunetics, Cambridge, MA), and 1000 μL of each suspension was placed into the extended slots of the MiniSlot 30 and fixed to the membrane by baking it at 120°C for 20 minutes. In each membrane, 28 samples were placed, and the last 2 channels of the MiniSlot 30 were reserved for placement of controls, containing a mixture of species of microorganisms that have been investigated by DNA probes at 2 concentrations (ie, 10^5 and 10^6) of bacterial cells. A Miniblotter 45

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