Endodontic Therapy Associated with Calcium Hydroxide As an Intracanal Dressing: Microbiologic Evaluation by the Checkerboard DNA-DNA Hybridization Technique

Carlos Alberto Soriano de Souza, DDS, MSc, Ricardo Palmier Teles, DDS, PhD, Renata Souto, MSc, Mario Augusto Escobar Chaves, DDS, and Ana Paula Vieira Colombo, DDS, PhD

Abstract

This study evaluated the predominant microbiota of infected necrotic pulps and the effects of calcium hydroxide therapy on these microorganisms by the checkerboard DNA-DNA hybridization technique. Conventional endodontic therapy associated with calcium hydroxide as intracanal dressing was performed in 12 single-rooted teeth with pulp necrosis and periradicular bone lesion. Samples were collected from the canal at baseline and 14 days after therapy, and the presence of 44 bacterial species was determined by the checkerboard method. Significant differences in the microbiota from baseline to post-therapy were sought by the paired-samples *t* test. The most prevalent species included *F. nucleatum ss. vincentii*, *C. sputigena*, *C. ochracea*, *S. constellatus*, *V. parvula*, *P. gingivalis*, *P. melaninogenica*, and *S. sanguis*. Most of the microorganisms were reduced after treatment, particularly *A. gerencseriae, A. israelii, A. naeslundii, C. gingivalis, C. ochracea, P. gingivalis, S. noxia, S. sanguis*, and *S. oralis* ($p < 0.05$). Conversely, *A. actinomycetemcomitans*, *C. sputigena*, and *E. corrodens* increased in numbers after therapy. These results indicate that conventional endodontic therapy with calcium hydroxide results in the reduction of pathogenic species associated with pulp necrosis. However, its use is limited, because it did not eliminate the whole spectrum of microorganisms.

Dr. Soriano de Souza and Renata Souto are affiliated with the Institute of Microbiology, Federal University of Rio de Janeiro, Brazil. Dr. Palmier Teles is affiliated with the Department of Periodontology, Forsyth Dental Center, Boston, Massachusetts, USA. Dr. Escobar Chaves is affiliated with the Department of Endodontics, Brazilian Navy, Rio de Janeiro, Brazil. Dr. Colombo is affiliated with the Institute of Microbiology, Federal University of Rio de Janeiro, Brazil.

Address reprint requests to Dr. Carlos Alberto Soriano de Souza, Rua Dias Ferreira, 541/Apt. 504, Rio de Janeiro, RJ, Brazil. E-mail: soso@osite.com.br.

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Over the years, numerous investigations have demonstrated that microorganisms and their products play a fundamental role in the pathogenesis of pulp and periapical infections (1, 2, 3). Cultural studies of primary endodontic infections have shown that these infections are polymicrobial, with a predominance of obligate anaerobic bacteria, including species of the genera *Eubacterium, Fusobacterium*, *Peptostreptococcus, Porphyromonas*, and *Prevotella* (1, 2, 3).

Nevertheless, recent advances in molecular biology techniques have led to a new perspective and a redefinition of the microbiota associated with endodontic infections. Consequently, fastidious and difficult-to-cultivate species, such as *Tannerella forsythensis (Bacteroides forsythus*) and *Treponema* spp., have been detected more frequently in samples from canal with pulp necrosis (4, 5).

Based on this evidence, the main goal of endodontic therapy has been focused on the elimination, or at least a significant reduction of, microorganisms present in the root canal system, which can be achieved mostly by the chemomechanical preparation of the conduct. However, studies have demonstrated that bacteria may be viable in the root canal even after vigorous mechanical instrumentation (6), leading to persistent or secondary intraradicular infections and therefore to treatment failure. Therefore, the use of an intracanal medication with antimicrobial activity between therapy sessions has been recommended to eliminate possible persistent microorganisms (7), particularly in cases of pulp necrosis with periradicular bone loss (8). Among the available intracanal dressings, calcium hydroxide is the most indicated and frequently used in the clinical practice (9). Its antibacterial properties are related to its high alkalinity, which results in the inactivation of bacterial membrane enzymes (10). Despite good clinical outcome (11), the use of calcium hydroxide as an intracanal medication may be limited by the presence of resistant species such as *Enterococcus faecalis*, a species particularly associated with persistent infection and treatment failure (12). Therefore, the present investigation aimed to determine the predominant microbiota of teeth with pulp necrosis and periradicular lesion and to evaluate the effects of endodontic therapy associated with calcium hydroxide on these microorganisms by whole genomic DNA probes and the checkerboard DNA-DNA hybridization method.

Materials and Methods

Study Participants

In an initial protocol, this investigation proposed the distribution of the study participants into two groups: a test group, treated with the calcium hydroxide intracanal medication, and a control group without this medication. However, for ethical reasons the inclusion of this control group was not approved by the Review Committee for Human Subjects. Therefore, this study focused only on the effects of the endodontic therapy associated with calcium hydroxide medication on the endodontic microbiota. Twelve systemically healthy adult patients (mean age 37 $\pm$ 0.8 years, range 21–82 years; 58% men) having a single-rooted tooth with necrotic pulp and radiographic evidence of periradicular bone loss were selected from the Clinic of Endodontics at the Odontoclinica Central da Marinha, Rio de Janeiro. None of the patients had experienced spontaneous pain or received antibiotics or root canal treatment of the affected tooth in the 3 months preceding entry into the study. In addition, teeth with deep periodontal

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pockets and/or that could not be isolated were excluded. In order to participate in the study, all patients were informed about its nature, and a signed consent form was obtained from each individual. The study protocol was approved by the Review Committee for Human Subjects of the Hospital Universitário Clementino Fraga Filho, Federal University of Rio de Janeiro.

Endodontic Treatment

After isolation of the tooth and access to the root canal, an initial bacteriologic sample was taken as described in Specimen Sampling, below. The root canals were cleaned and shaped by the step-down technique (13), using hand files and Gates-Glidden drills (Dentsply/Maillefer, Ballaigues, Switzerland) with 5.25% sodium hypochlorite irrigation. Then the canals were dried with sterile paper points and filled with a paste of calcium hydroxide (Dentsply Herpo, Petrópolis, RJ, Brazil) and saline solution in a creamy consistency by means of Lentulo spiral (Dentsply/Maillefer). The coronal cavities were sealed with a temporary filling, Coltosol (Vigodent, São Paulo, SP, Brazil). In all cases, the calcium hydroxide paste was left in the canals as a dressing for 14 days. After that, the dressing was removed by irrigation with saline solution, and the second bacteriologic samples were taken. The canals were then filled with gutta-percha points and cement (Endo Fill, Dentsply Herpo), using the lateral condensation technique.

Specimen Sampling

Samples were obtained from root canals using strict asepsis in a procedure previously described (14). Briefly, the tooth was isolated with the rubber dam, and a cotton applicator was used to clean the surface of the tooth and surrounding field with 3% hydrogen peroxide, followed by decontamination with 5.25% sodium hypochlorite. Complete access preparations were made with sterile burs without water spray. If the root canal was dry, a small amount of sterile saline solution was introduced into the canal. Samples were initially collected by means of a size 15 K-type file (Dentsply/Maillefer) introduced to a level approximately 1 mm short of the tooth radiographic apex, and a discrete filing motion was applied. Then, two sequential sterile paper points were placed to the same level and used to soak up the fluid in the canal for 1 minute. Both paper points were transferred to Eppendorf tubes containing 1 ml of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). The second sample was taken in the same way, right after removal of the calcium hydroxide dressing and before the root canal filling.

Microbiologic Assessment

The presence and levels of 44 bacterial species (Table 1) were determined by a modification of the checkerboard DNA-DNA hybridization method described by Socransky et al. (15). In brief, the samples collected were placed in separate Eppendorf tubes, the cells were lysed, and denatured DNA was fixed in individual lanes on a nylon membrane (Hybond N+; Amersham Biosciences do Brasil, São Paulo, Brazil), using the slot blot device Minislot 30 (Immunetics, Cambridge, MA, USA). Forty-four digoxigenin-labeled (Roche Applied Science, Indianapolis, IN, USA) whole genomic DNA probes were constructed and hybridized perpendicularly to the lanes of the clinical samples using the Miniblotter 45 (Immunetics). Bound probes were detected using phosphatase-conjugated antibody to digoxigenin (Roche Applied Science) and chemiluminescence (CDP-Star; Amersham Biosciences). Signals were evaluated visually by comparison with the standards at 10^5 and 10^6 bacterial cells for the test species on the same membrane. They were recorded as follows: 0, not detected; 1, $<10^5$ cells; 2, ~10^5 cells; 3, 10^5 to 10^6 cells; 4, ~10^6 cells; and 5, $>10^6$ cells. The sensitivity of this assay was adjusted to permit detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe. This procedure was carried out to provide the same sensitivity of detection for each probe. Failure to detect a signal was recorded as zero, although counts in the 1 to 10,000 range could conceivably have been present.

Statistical Analysis

The microbiologic data were expressed as a percentage of positive samples (prevalence) and mean counts $\times 10^5$ (level) of each species. In the analysis of prevalence, only the absence or presence of the mi-

TABLE 1. Whole genomic DNA probes for 44 bacterial species tested against root canal samples of pulp necrosis by the checkerboard DNA-DNA hybridization technique.

Species	Strains	Species	Strains
Actinobacillus actinomycetemcomitans (sorotype a)	43718*	*Leptotrichia buccalis*	14201*
Actinobacillus actinomycetemcomitans (sorotype b)	29523*	*Neisseria mucosa*	19696*
Actinomyces gerencseriae	23860*	*Peptostreptococcus micros*	33270*
Actinomyces israelii	12102*	*Porphyromonas endodontalis*	35406*
Actinomyces naeslundii genospecies 1	12104*	*Porphyromonas gingivalis*	33277*
Actinomyces odontolyticus	17929*	*Prevotella intermedia*	25611*
Actinomyces viscosus	43146*	*Prevotella melaninogenica*	25845*
Campylobacter gracilis	33236*	*Prevotella nigrescens*	33563*
Campylobacter rectus	33238*	*Propionibacterium acnes I*	11827*
Campylobacter showae	51146*	*Propionibacterium acnes II*	11828*
Capnocytophaga ochracea	33596*	*Selenomonas noxia*	43541*
Capnocytophaga gingivalis	33624*	*Streptococcus anginosus*	33397*
Capnocytophaga sputigena	33612*	*Streptococcus constellatus*	27823*
Eikenella corrodens	23834*	*Streptococcus gordonii*	10558*
Enterococcus faecalis‡	29212*	*Streptococcus intermedius*	27335*
Eubacterium nodatum	33099*	*Streptococcus mitis*	49456*
Eubacterium saburreum	33271*	*Streptococcus oralis*	33037*
Fusobacterium nuc ss. nucleatum	25586*	*Streptococcus sanguis*	10556*
Fusobacterium nuc.ss. polymorphum	10953*	*Tannerella forsythensis*	43037*
Fusobacterium nucleatum ss. vincentii	49256*	*Treponema denticola*	B1†
Fusobacterium periodonticum	33693*	*Treponema socranskii*	S1†
Gemella morbillorum	27824*	*Veillonella parvula*	10790*

* ATCC (American Type Culture Collection, Rockville, MD).

† FDC (Forsyth Institute, Boston, MA).

‡ Species not classified as a member of the oral microbiota.

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