Scientific Article

Antibacterial Effects of Chemomechanical Instrumentation and Calcium Hydroxide in Primary Teeth With Pulp Necrosis

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Abstract: ***Purpose:*** *This study's purposes were to: evaluate the antibacterial effect of chemomechanical instrumentation and a calcium hydroxide-based dressing in primary teeth with pulp necrosis secondary to trauma; and detect the presence of Fusobacterium nucleatum and black-pigmented rods in the canals of these teeth.* ***Methods:*** *Microbiological sample collections (MSCs) were obtained: after coronal access to the canals of primary incisors (in 18 teeth; MSC 1); after chemomechanical instrumentation (in 10 teeth; MSC 2); and 72 hours after removal of intracanal medication (in 18 teeth; MSC 3). These samples were sent for microbiological processing. The results were analyzed statistically via chi-square, analysis of variance, and Games-Howell tests (P<.05).* ***Results:*** *Micro-organisms were isolated in approximately 94%, 10%, and 83% of canals, respectively, in MSCs 1, 2, and 3. There was a statistically significant difference only between MSCs 1 and 2 (P<.03). F nucleatum and black-pigmented rods were detected in approximately 56% and 11% of canals in MSC 1, respectively.* ***Conclusion:*** *Chemomechanical instrumentation and calcium hydroxide-based dressing have an antibacterial effect by significantly reducing the number of micro-organisms in the main root canal. They showed a limited efficacy, however, and did not prevent bacterial regrowth after endodontic therapy in primary teeth with pulp necrosis secondary to trauma. (Pediatr Dent 2011;33:307-11)* *Received October 21, 2009 / Last Revision March 19, 2010 / Accepted April 22, 2010*

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Pulp necrosis has been reported as one of the most common sequelae in the primary dentition after trauma.[1,2] Endodontic treatment is indicated in cases where necrosis is associated with coronal discoloration and is accompanied by alterations that indicate the presence of infection, such as internal, inflammatory, or replacement root resorption and periapical inflammation or fistula.[3-5]

After necrosis, the microenvironment of the pulp space becomes propitious to factors that influence microbial colonization and multiplication. Anaerobic bacteria, such as black-pigmented rods (**BPRs**) and *Fusobacterium nucleatum*, are the most predominant micro-organisms in teeth with endodontic infection.[6-8] BPRs are frequently found in human primary teeth with necrotic pulps and apical periodontitis,[6-8] while *F nucleatum* has been reported as the most commonly isolated bacterial strain in root canals of permanent teeth with periapical lesions and intact pulp chambers.[9]

The success of root canal therapy depends directly on reducing or eliminating the endodontic microbiota, and chemomechanical instrumentation is one of the most important phases of the endodontic treatment.[10] The bacteria present in the root canal system, however, may survive, grow, and multiply, even after biomechanical preparation, if an interappointment intracanal medication is not used. The use of an intracanal dressing has been shown to be a valuable aid in root canal system disinfection, eliminating residual pathogens and neutralizing their toxic products to create a favorable environment for periapical healing to occur.[10]

Calcium hydroxide is routinely used in endodontics as an intracanal medication due to its favorable properties. These include: biocompatibility[11,12]; antibacterial activity by enzymatic inhibition; bacterial cell wall alteration[10,13] and inactivation of bacterial endotoxin[14,15]; anti-inflammatory activity; and repair action by activation of alkaline phosphatase, which is an enzyme involved in the induction of bone tissue formation.[13]

The purposes of the present study were to: evaluate the antibacterial effect of chemomechanical instrumentation associated with the use of a calcium hydroxide-based dressing in primary teeth with pulp necrosis secondary to trauma; and detect the presence of *Fusobacterium nucleatum* and **BPRs** in the canals of these teeth.

Methods

This study was a cross-sectional investigation involving clinical and laboratory procedures. Eligible participants were selected from 2- to 6-year-old boy and girl patients who had been referred for dental treatment at the Pediatric Dentistry Clinic of the School of Pharmacy, Dentistry and Nursing,

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Federal University of Ceará, Fortaleza, Ceará, Brazil, between July 2007 and October 2008 and who presented at least 1 primary anterior tooth (central and/or lateral incisors) with pulp necrosis secondary to traumatic injury. Fulfillment of the following inclusion criteria was required: tooth crown discoloration associated with evidence of endodontic infection (fistula, periapical lesion, and/or external root resorption); no systemic alterations or use of antibiotics or antimicrobials within the previous 3 months; teeth with less than two thirds of root resorption; and no previous root canal treatment.

Eighteen patients who met all of these inclusion criteria were enrolled, providing a total of 22 teeth. The study purposes were fully explained to the parents/guardians, who signed a written informed consent form authorizing their children's enrolment in the study. The research protocol was reviewed and approved by the Institutional Research Ethics Committee Medical School of the Federal University of Ceará, Fortaleza, Ceará, Brazil (process no. 105/07).

Excluded from the study were children who: used antibiotics or antimicrobial mouthwashes during the course of the trial; had an uncooperative behavior that impeded microbiological sample collection (**MSC**); missed a clinical appointment; or lost the provisional coronal restoration between the treatment sessions.

Clinical procedures. All clinical procedures of the MSC were performed by 2 trained, experienced operators. The clinical procedures of MSC were performed by 2 graduate students with clinical experience attending the postgraduate program in pediatric dentistry. Before the beginning of the study, the operators participated in a pilot study collecting microbiological samples from 5 subjects (not included in the study) for training and calibration purposes to ensure standardization of all clinical steps.

After infiltrative local anesthetic, a rubber dam was placed, and the tooth surface was cleaned with 2.0% chlorhexidine digluconate. Coronal access to root canals was prepared with air/water cooled high-speed spherical diamond burs (KG Sorensen Indústria e Comércio, São Paulo, São Paulo, Brazil) followed by tapered, safe-ended steel burs (Batt burs, Maillefer Instruments, Ballaigues, Switzerland). The pulp chamber was irrigated with sterile saline and dried. Root canal length was determined radiographically. The first MSC (**MSC 1**) was done immediately after these procedures. Three sterile absorbent paper points of a size compatible with the root canals were sequentially introduced into the canals up to the radiographic apex level under aseptic conditions. After approximately 1 minute, the paper points were removed, transferred to Eppendorf tubes containing 1 mL of reduced transport fluid (**RTF**), and taken from the laboratory soon after for microbiological processing.

After MSC 1, chemomechanical instrumentation of the canals was done according to the progressive neutralization technique using a sequence of 3 K-files (Maillefer Instruments), compatible with the canal size and irrigation, with 2 mL of 0.5% sodium of hypochlorite at each change of file followed by suction of the solution. The working length was established 1 mm short of the radiographic apex. The canals were dried with sterile absorbent paper points and filled with 17% ethylenediaminetetraacetic acid (OdahcanHerpo Produtos Dentários Ltda, Rio de Janeiro, Brazil) for 3 minutes. Next, the canals were flushed with 2 mL of sterile saline, suctioned, and dried with sterile absorbent paper points.

After chemomechanical instrumentation, a new disinfection of the operative field was performed with 2.0% chlorhexidine digluconate and the second MSC (**MSC 2**) was done in 10 teeth similarly to MSC 1 and sent for microbiological analysis. The root canals were then filled with a calcium hydroxide-based paste (Calen, SS White Artigos Dentários Ltda, Rio de Janeiro; composition=2.5 g calcium hydroxide, 0.5 g zinc oxide, 0.05 g colophony, and 1.75 mL polyethylene glycol 400 [vehicle]) using a special syringe followed by lentulo spirals. Complete filling of the canals was confirmed radiographically. The pulp chambers were cleaned and the access cavities were sealed with conventional glass ionomer cement (Vidrion R, SS White Artigos Dentários Ltda).

After 30 days, the tooth surfaces were cleaned with 2.0% chlorhexidine digluconate, under rubber dam isolation and the coronal seals were opened. The intracanal medication was removed with a K-file, and the canals were gently flushed out with sterile saline, dried, and left empty. The access cavities were sealed again with conventional glass ionomer cement. After 72 hours, the tooth surfaces were cleaned again with 2.0% chlorhexidine digluconate under rubber dam isolation, and the third MSC (**MSC 3**) was collected similarly to MSC 1 and sent for microbiological analysis.

Once the sample collection phase was completed, all canals were obturated with a zinc oxide and eugenol-based cement (Biodinâmica Quimica e Farmaceutica Ltda, Ibipora, Paraná, Brazil).

Microbiological processing. The examination of all microbiological samples was done by a single blinded experienced microbiologist.

The tubes containing the microbiological samples were vortexed for approximately 2 minutes for homogenization of the inocula, which were serially diluted in phosphate buffer saline (**PBS**) until reaching 10^{-1} to 10^{-5} final concentrations and seeded in triplicate in Petri dishes containing Brain Heart Infusion agar (Difco Laboratories, Detroit, Mich) supplemented with cysteine, hemin, menadione, and defibrinated sheep blood for detection of anaerobic bacteria, using calibrated pipettes. The dishes were placed in an anaerobiosis jar containing an atmosphere generator (Probac, São Paulo) and were incubated at 37°C for 7 days. Thereafter, the bacterial colonies were examined with a stereomicroscope at 2.5× magnification (Jena, Germany) regarding their size, color, density, shape, elevation, and consistency, and with an optical microscope at 100× magnification (Alphaphet-2 YS2, Nikon, Tokyo, Japan) for the gram staining. A respiratory test was done, and the number of colony-forming units (**cfu**) per mL of inoculum was counted.

Identification of *F nucleatum* and BPRs was done by the pathognomonic characteristics observed in the dishes and gram staining.

Statistical analysis. The data were entered in Excel 7.0 (Microsoft Corp, Redmond, Wash) and SPSS 11.5 (SPSS Inc, Chicago, Ill) for Windows software. The chi-square test was used to analyze the statistical significance of the differences with $P<.05$. Analysis of variance and Games-Howell multiple-comparison tests were used to compare the cfu counts obtained in MSCs 1, 2, and 3 and verify the existence of differences among the MSCs.

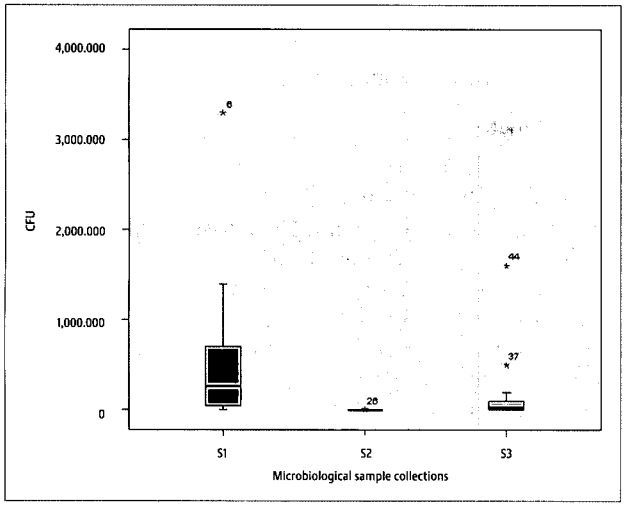

Figure 1. Graphic presentation of the mean colony-forming unit counts in microbiological sample collections 1, 2, and 3 and the differences among them.

Results

Four patients (4 teeth) were excluded due to sample contamination during the course of the study (contamination of the root canal due to loss of the provisional coronal seal or contamination of the operative field during the collection procedures) or failure to attend all clinical appointments. This reduced the number of teeth from 22 to 18.

After coronal access to the canals (MSC 1), micro-organisms were isolated in 17 of 18 (~94%) root canals, with a mean cfu count of 5.4×10^5. After chemomechanical instrumentation (MSC 2), micro-organisms were detected in only 1 of 10 (10%) root canals, with a mean cfu count of 4.3×10^2. After removal of the intracanal medication (MSC 3), micro-organisms were isolated in 15 out of 18 root canals, with a mean cfu count of 1.5×10^5.

There was a statistically significant reduction ($P<.03$) in the cfu counts between MSCs 1 and 2, but no statistically significant difference was found between MSCs 1 and 3 ($P<.17$) or between MSCs 2 and 3 ($P>.22$). The mean cfu counts in MSCs 1, 2, and 3 and the differences among the 3 MSCs are graphically illustrated in Figure 1.

Table 1 shows the distribution of bacterial morphotypes in MSCs 1, 2, and 3. In MSC 1, there was a predominance of gram-negative cocci (15/18; 83.3%) and gram-negative bacilli (14/18; ~78%). In MSC 2, gram-positive cocci were the only morphotype detected in the canals after instrumentation (1/10; 10%). In MSC 3, there was a reduction in the counts of all morphotypes, except for gram-positive cocci. For all analyzed morphotypes, there was a substantial reduction in the bacterial counts from MSC 1 to 2 ($P>.01$) and an increase in the bacterial counts from MSC 2 to 3 ($P=.006$).

F nucleatum and black-pigmented anaerobic rods were: observed in approximately 56% (10/18) and 11% (2/18) of the MSC 1 canals, respectively; not detected in MSC 2; and present in approximately 17% (3/18) and 6% (1/18) of the MSC 3 canals, respectively.

Discussion

In the present study, bacteria were found in approximately 95% of the initial samples collected from the root canals after coronal opening. These results are similar to those of previous studies,[6,16-18] which reported the presence of bacteria in the initial sample collection in 92%[16] to 99%[18] of the canals. A possible explanation for not obtaining 100% of positive canal samples in the first microbiological collection, as observed by other authors,[6,19,20] is the fact that bacteria may be harbored at different dentin depths and sometimes can only be detected after instrumentation.[18] According to Ferrari et al.,[16] bacteriological sampling procedures and laboratorial culture processing may not provide an accurate reflection of the root canal microbiota because several types of micro-organisms fail to survive for identification under regular laboratory conditions.

Chemomechanical instrumentation was capable eliminating micro-organisms from 90% of the cases in the present study. Bacterial reduction rates of approximately 100%,[21] 76%,[22] and 50% to 100%[20] have been reported after instrumentation. According to Peters et al.,[22] the differences observed in the reduction in the number of micro-organisms after instrumentation may be related to the different sodium hypochlorite concentrations and irrigation systems used in each study. A 0.5% sodium hypochlorite solution was used in the present investigation, while other studies previously mentioned used concentrations of 2.5%[20,21] and 2%.[22]

In the present study, MSC was collected in only 10 teeth for MSC 2, because a statistically significant reduction in the cfu counts between MSC 1 (coronal access to the canals) and MSC 2 (after chemomechanical instrumentation) was obtained with this sample size, confirming the findings of Soares.[23] According Soares, chemomechanical instrumentation promotes a partial and temporary antisepsis, regardless of the instrumentation technique and concentration of the irrigating solutions. This is due to the following factors:

1. the irregular morphology of the root canal system, which is the reason why some regions are inaccessible to instruments and chemical irrigants, and micro-organisms that remain lodged in anfractuous niches of the dentin walls;
2. the diffuse nature of the endodontic infection, which is the reason why the intracanal medications do not have an immediate action in all regions of the canal space; and

Table 1. PERCENTAGE OF BACTERIAL MORPHOTYPES IDENTIFIED IN THE ROOT CANALS OF PRIMARY TEETH WITH PULP NECROSIS SECONDARY TO TRAUMA

Bacteria	MSC* 1 (N=18)	MSC 2 (N =10)	MSC 3 (N =18)
	% (N)	% (N)	% (N)
Gram-positive cocci	67 (12)	10 (1)	67 (12)
Gram-negative cocci	83 (15)	0 (0)	61 (11)
Gram-positive bacilli	44 (8)	0 (0)	22 (4)
Gram-negative bacilli	78 (14)	0 (0)	33 (6)
Fusobacterium nucleatum	56 (10)	0 (0)	17 (3)
Black-pigmented rods	11 (2)	0	6 (1)
Colony-forming units†	5×10^5	4×10^2	2×10^5
Positive samples	94 (17)	10 (1)	83 (15)

* MSC=microbiological sample collection.

† Mean; MSC 1, 2, and 3 correspond to the microbiological samples collected after coronal opening, root canal instrumentation, and use of intracanal medication, respectively. Four patients (4 teeth) were excluded from the study due to sample contamination or failure to attend all clinical appointments, thus reducing the number of teeth from 22 to 18.

3. presence of blood, exudate, and tissue remnants.

It was expected that, after use of a calcium hydroxide-based intracanal dressing for 30 days, the endodontic microbiota would be similar to or smaller than the one found after chemomechanical instrumentation. In some cases, however, there was an increase in the cfu counts after use of the intracanal medication compared to both the first and the second MSCs, as observed in previous studies.[21,22,24] According to Siqueira and Lopes,[24] bacteria may survive after use of intracanal medication for several reasons:

1. Bacterial strains present in infected root canals may be intrinsically resistant to the medicament used.
2. Bacteria may be enclosed within anatomical variations inaccessible to the intracanal dressing.
3. The medicament may be neutralized by tissue components and by bacteria or their products and by products, losing its antibacterial effects.
4. Medicaments may remain in the root canal system for an insufficient time to reach and kill the bacterial cells.
5. Bacteria may alter their pattern of gene expression after changes in the environmental conditions, which may allow them to survive even under unfavorable conditions.

The increase of cfu counts using an intracanal medication may also be associated with the contamination of the root canal system between the treatment sessions due to the formation of visually imperceptible cracks in the temporary coronal seal[16,21] or dentin buffering capacity, which reduces the diffusion of hydroxyl ions through the root dentin, a factor that is directly associated with the success of calcium hydroxide as an intracanal medication.[13]

It should be emphasized that, in the present study, the efficacy of the intracanal dressing was evaluated 72 hours after removing the calcium hydroxide paste and leaving the canals empty and sealed coronally, as recommended by Soares et al.[25] According to those authors,[25] within this period without medication, the micro-organisms remaining in the main root canal and in the canal ramifications proliferate and recolonize the root canal walls, possibly reaching counts equivalent to those found before the endodontic treatment. Therefore, the samples obtained from the canals under these conditions represent the actual microbiological conditions found in the root canal system. There was no need, however, to leave the canals empty for microbiological sampling after instrumentation, as recommended by Soares et al.[25] This is because the antibacterial action of the chemomechanical instrumentation is limited to the main canal and does not act on bacteria lodged in canal system ramifications.

There was a predominance of gram-positive cocci after use of the intracanal medication, which is consistent with the results of previous studies.[16,18,21,26,27] According to De Paz,[27] gram-positive bacteria can modify their nutritional demands in periods of starvation, limiting the required amount of nutrients to save the energy used for metabolism. This mechanism enables their survival for long periods of time. In addition, alkalotolerant bacterial species may survive in the canal space after placement of calcium hydroxide dressings, suggesting a capability to adjust to alkaline shifts. These species can adapt to alkaline environments by maintaining a homeostasis between the external and intracellular pH. Bacteria also can be organized as biofilms and protect themselves mutually.

F nucleatum was detected in approximately 56% of the initial samples. This result is consistent with the findings of other studies,[9,20] which reported that this micro-organism is highly prevalent in teeth with apical periodontitis and intact pulp chambers, while other authors found a lower prevalence (<12%[26] and 12%[22]). The results of those studies refer to permanent teeth, however, since there is no report in the literature associating this micro-organism having endodontic microbiota in primary teeth with pulp necrosis and intact crown.

BPRs were detected in only approximately 11% of the initial samples. This finding contrasts with the results of 2 previous studies,[6,7] which reported a higher prevalence of these micro-organisms in the root canals of primary teeth with necrotic pulps and apical periodontitis (30% and ~36%, respectively). Those authors evaluated teeth with carious lesions, however, while in the present study pulp necrosis developed after a traumatic injury, which may explain this difference in the prevalence.

In a study using checkerboard DNA-DNA hybridization to determine the microbiota of root canals of human primary teeth, Ruviére et al.[8] verified a great bacterial diversity, characterizing a polymicrobial endodontic infection with presence of anaerobic and facultative micro-organisms, black-pigmented rods, and streptococci. A large number of anaerobic species were detected in teeth with necrotic pulp and apical periodontitis, and a significantly smaller number of bacterial cells were found in teeth with irreversible pulpitis.

According to Sundqvist,[9] selective pressures act inside the root canals, favoring the growth and multiplication of certain bacteria, and limiting the survival of other bacterial species. These pressures may be:

1. Oxygen tension: Due to oxygen consumption and the subsequent development of a low oxidation-reduction potential, there is a proportional reduction in the number of facultative bacteria concomitantly with an increase in the number of anaerobic bacteria.
2. Availability of nutrients: Root canal conditions are favorable to the growth of anaerobic bacteria capable of fermenting amino acids and peptides, while the number of bacteria that obtain energy mainly from carbohydrate fermentation is reduced due to the lack of viable nutrients.
3. Bacterial inter-relationship: Bacteria establish an alimentary chain interchange, and their presence in groups in the root canals is not a mere coincidence.

Conclusions

Based on this study's findings, the following conclusions can be made:

1. Chemomechanical root canal treatment promoted quantitative and qualitative changes in the resident endodontic microbiota of primary teeth with pulp necrosis secondary to trauma, with an overall reduction in the number of micro-organisms at the end of the treatment.
2. Specifically, the greatest decrease of colony-forming unit counts was observed among gram-negative bacteria, while the gram-positive bacteria were more resistant.

3. Chemomechanical instrumentation and calcium hydroxide-based dressing had a limited efficacy and did not prevent the regrowth of bacteria, especially some gram-positive coccal species, after endodontic therapy.
    - *Fusobacterium nucleatum* and black-pigmented anaerobic rods were presents in primary teeth with pulp necrosis secondary to trauma.

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