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ANTIBACTERIAL EFFECT OF ROOT CANAL PREPARATION AND CALCIUM HYDROXIDE PASTE (CALEN) INTRACANAL DRESSING IN PRIMARY TEETH WITH APICAL PERIODONTITIS

EFEITO ANTIBACTERIANO DO PREPARO BIOMECÂNICO E DO CURATIVO DE DEMORA COM PASTA À BASE DE HIDRÓXIDO DE CÁLCIO (CALEN) EM DENTES DECÍDUOS COM LESÃO PERIAPICAL

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ABSTRACT

The aim of this study was to evaluate the antibacterial action of root canal mechanical preparation using 2.5% sodium hypochlorite as the irrigating solution and a calcium hydroxide paste as the antibacterial intracanal dressing in human primary teeth root canals with pulp necrosis and apical periodontitis by means of microbial culture. A total of 26 root canals of human primary teeth with pulp necrosis and apical periodontitis were used. Samples were collected before, 72h after biomechanical treatment and 72h after removal of the intracanal dressing. Comparison by Wilcoxon test showed that root canal mechanical preparation effectively eliminated all microorganisms in 20% of the root canals, and the intracanal dressing in 62.5%; however, the cumulative action of biomechanical treatment and intracanal dressing eliminated the microorganisms of 70% of the root canals ($p<0.001$). Isolated root canal mechanical preparation presented poorer microbiological results that those obtained with root canal mechanical preparation and the use of an intracanal dressing indicating the necessity of topical application of an intracanal medication between sessions in primary teeth with pulp necrosis and apical periodontitis.
Uniterms: Tooth, deciduos; Apical periodontitis; Root canal preparation; Calcium hydroxide.

RESUMO

O objetivo do presente estudo foi avaliar, por meio de cultura bacteriológica, a ação antibacteriana do preparo biomecânico utilizando como solução irrigadora o hipoclorito de sódio a 2,5% e da pasta Calen utilizada como curativo de demora em canais radiculares de dentes decíduos de humanos com necrose pulpar e lesão periapical. Foram selecionados 26 dentes decíduos de humanos portadores de necrose pulpar e lesão periapical. As colheitas microbiológicas foram efetuadas antes e 72 horas após o preparo biomecânico e 72 horas após a remoção do curativo de demora. A comparação por meio do teste de Wilcoxon mostrou que o preparo biomecânico foi eficaz na eliminação dos microrganismos dos canais radiculares em 20% dos casos e o curativo de demora em 62,5%, enquanto que a ação cumulativa do preparo biomecânico e do curativo de demora eliminou os microrganismos em 70,0% dos casos ($p<0.001$). Pôde-se concluir que o preparo biomecânico, isoladamente, apresentou resultados microbiológicos inferiores àqueles obtidos quando o mesmo foi associado ao curativo de demora, mostrando a necessidade de aplicação tópica de um curativo de demora entre sessões em dentes decíduos portadores de necrose pulpar e lesão periapical.
Unitermos: Dente decíduo; Periodontite periapical; Preparo de canal radicular; Hidróxido de cálcio.

INTRODUCTION

The success of endodontic treatment depends on many factors, however the decrease or elimination of bacterial infection is one of the most important[19].

In chronic endodontic infections, bacteria and their by-products are present not only in the main root canal but also disseminated throughout the root canal system in both primary[2] and permanent teeth[6,7].

One of the aims of root canal mechanical preparation associated with irrigating solutions[4,11,15] in the endodontic treatment of primary teeth is to eliminate infection. However, because microorganisms are not only in the main root canal[5], root canal mechanical preparation is not efficient for elimination[15]. Therefore, substances must be used to fight the infection in the deep and diffused regions throughout the dentine, which are inaccessible to root canal mechanical preparation and the patient's defense system[5-7].

Numerous substances have been used as intracanal dressings between sessions in primary teeth with pulp necrosis, such as formocresol, camphorated paramonochlorophenol and calcium hydroxide[3,9,11,12,15]. However, there are few studies in the literature evaluating the efficacy of these medicaments upon the main microorganisms found in the root canals of primary teeth with pulp necrosis[12,16,17].

Thus, the aim of this study was to evaluate, *in vivo* by microbial culture, the antibacterial action of root canal mechanical preparation associated with irrigating solution and a calcium hydroxide paste used as an intracanal dressing in root canals of human primary teeth with pulp necrosis and apical periodontitis.

MATERIALS AND METHODS

The participants of this study were 26 patients (3-7 years old) of both genders selected consecutively from patients treated at the Pediatric Clinic of the Ribeirão Preto Dental School - USP. They were in good general health and had not been treated with antibiotics for at least 3 months. This study was approved by the Faculty Ethics Committee (nº. 2000.1.92.58.6).

A total of 26 primary teeth (8 root canals from maxillary incisors, 8 root canals from 8 mandibular molars and 10 root canals from 10 mandibular molars) with necrotic pulp and radiographically visible radiolucent areas in the region of the bone furcation and/or the periapical region were used. The teeth had carious lesions but the coronary structure permitted the isolation of the surgical area with a rubber dam and later restoration. They also had intact roots or at least 2/3 of preserved root, no periodontal pockets and no previous intervention of the root canals.

Clinical procedures

After antisepsis of the oral cavity by rinsing for 1 min with 10.0mL of 0.12% digluconate chlorhexidine (Periogard, Colgate-Palmolive Ind. Brasileira, Osasco, Brazil), local anesthesia was applied and a rubber dam was placed. The surgical field was then disinfected with 1% digluconate chlorhexidine.

After removal of the carious tissue, the area was disinfected and root canal access was done with high-speed round diamond burs (KG Sorensen Indústira e Comércio, São Paulo, Brazil) and Endo-Z files (Les Fills d'August, Maillefer, Ballaigues, Switzerland), cooled with air and water.

The first bacteriological sample was collected just after crown access introducing 4 sequential sterile absorbent paper points (Tanari Industrial Ltda., Manaus, Brazil), of a number compatible with the root canal diameter. After 30s, the paper points were removed from each canal and were placed in a test tube containing 2.0mL of reduced transport fluid (RTF) prepared according to Syed and Loesche[14] and processed microbiologically.

For the maxillary molars samples were collected from the palatal root canal for the maxillary molars and from the distal for the mandibular molars.

In the same session, neutralization of the septic-necrotic content of the root canal was performed using K files (Les Fills d'August, Maillefer, Ballaigues, Switzerland) associated with 2.5% sodium hypochlorite as irrigant solution. Working length was then performed at 1mm from the radiographic apex or at the limit of the root resorption level. Root canal mechanical preparation was performed with 4 K-files and instrumentation was followed by irrigation with 1.8mL of 2.5 % sodium hypochlorite, between each instrument.

The root canals were then dried with sterile absorbent paper points, a sterile cotton pellet was placed at the entrance of the root canal and the coronary opening was sealed with Cimpat (Spécialités Septodont, Saint Maur, France) and zinc oxide and eugenol cement (IRM, Dentsply Indústria e Comércio Ltda., Petrópolis, Brazil) as double coronary sealing.

After 72h, a second bacteriological sample was taken from the root canals such as the first one. The root canal was then irrigated with 1.8mL of sterile saline and dried. Ethylenediaminetetraacetic acid (EDTA, Odahcan Herpo Produtos Dentários Ltda., RJ, Brazil) was subsequently applied and agitated for 3 min with a K-file. The root canal was again irrigated with 1.8mL of sterile saline and dried with sterile absorbent paper points.

The root canals were then filled with Calen (S.S. White Artigos Dentários Ltda., Rio de Janeiro, Brazil) composed by calcium hydroxide – 2.5g, zinc oxide – 0.5g, colophony – 0.05g and polyethylene glycol 400 – 1.75mL, that was applied with a special syringe (ML, S.S. White Artigos Dentários Ltda, Rio de Janeiro, Brazil.) with a long needle (Gengibrás, 27 G Ibras CBO Ind. Bras., São Paulo, Brazil) to the working length. The complete filling of the root canal with the paste was confirmed radiographically and coronal sealing was performed as described previously.

The intracanal dressing was maintained in the root canal for 30 days and then removed by saline irrigation. The root canals were dried and followed by double coronary sealing. After the 72 h that the root canals remained empty, a third bacteriological sample was taken similar to the first one.

The root canals were then filled with Calen paste thickened with zinc oxide and the teeth were restored.

Laboratory Procedures

At the laboratory, 4-6 glass beads and a sterile metal wing were added to the test tubes containing the samples. The tubes were agitated for 2 min in a mixer (Mixtron-Toptronix, SP, Brazil) at maximum speed. Subsequently, serial decimal dilutions up to 10^{-5} were made in phosphate buffered saline (PBS) under laminar airflow. A volume of 0.05mL of the pure samples and of each dilution were seeded, with a sterile calibrated pipette, onto plates containing blood agar - Ba (Difco, Detroit, USA), Mitis Salivarius agar - Ms (Difco) and blood agar supplemented with 5.0µg/mL hemin and 1.0µg/mL menadione - Bak (Sigma Chemical Co., St. Louis, USA). Plates containing MacConkey agar - Mc (Difco, Detroit, USA) and bacitracin sucrose agar - SB_{20} (Difco, Detroit, USA) were seeded up to 10^{-1} dilutions. SB_{20} was prepared according to Davey and Rogers[2], modified by the substitution of sucrose with cane sugar, according to Torres[18].

To the remaining undiluted sample, 5.0 mL sodium thioglycolate was added (without glucose or pH indicator; Difco) in order to detect microorganisms present at levels less than or equal to 20 cfu/mL.

Bak plates were incubated anaerobically using the GasPak system for 7-10 days; Ms and SB_{20} plates microaerobically by the candle jar system for 2-3 days and the Ba and Mc plates aerobically for 24-48 h, at 37ºC. After incubation, colonies were counted with a stereomicroscope (Nikon, Tokyo, Japan) under reflected light and the cfu/mL was calculated.

Statistical Analysis

The results were analyzed statistically by the Wilcoxon nonparametric test using the software GMC 8.1 (http://www.forp.usp.br/restauradora/gmc/gmc.html).

RESULTS

Of the 26 root canals initially selected, only 20 samples remained at the end of the experiment, due to lack of patient return or to loss of the coronal seal.

As shown in Table 1, anaerobic microorganisms were present in all 20 root canals (100%). In 6 cases (30%), black-pigmented bacilli were found. Aerobic microorganisms were found in 12 root canals (60%) and streptococci were present in 17 root canals (85%). Mutans streptococci were found in 6 canals (30%) and aerobic Gram-negative bacilli were found

TABLE 1- Microorganisms (cfu) in human primary teeth root canals with pulp necrosis and periapical lesion

Case	Anaerobic	BPB	Aerobic	Streptococcus	MS	GNAB	Thiogly colate
1	13,300,000	71,000	0	0	0	0	+
2	3,600,000	315,000	0	0	0	0	+
3	9,900,000	440,000	40	0	0	0	+
4	5,350,000	10,200	0	1,130	0	0	+
5	1,260,000	0	7,000	23,000	40	0	+
6	2,400,000	0	183,000	192,000	0	0	+
7	6,400,000	0	72,000	120,000	0	40	+
8	301,000	0	1,870	1,370	0	0	+
9	3,230,000	101,000	0	20	0	0	+
10	710,000	49,000	0	370	0	0	+
11	140	0	0	40	0	0	+
12	4,000	0	0	120	0	0	+
13	4,100	0	40	80	0	0	+
14	3,800	0	0	2,400	20	0	+
15	140	0	80	120	0	0	+
16	580,000	0	38,000	149,000	134,000	0	+
17	570,000	0	3,100	137,000	1,690	40	+
18	2,800	0	200	2,500	660	40	+
19	8,500	0	2,600	2,800	0	0	+
20	720,000	0	2,600	2,200	140	0	+
20	(100%)	6 (30%)	12 (60%)	17 (85%)	6 (30%)	3 (15%)	20 (100%)

BPB = black-pigmented bacillus; MS = mutans streptococcus; GNAB = gram-negative aerobic bacillus; + = bacterial development

in 3 root canals (15%). Thioglycolate medium was positive in all cases.

As seen in Figure 1, the prevalence of microorganisms in the root canals after root canal mechanical preparation greatly decreased. Anaerobic microorganisms were eliminated in 7 samples (65% reduction) ($p<0.001$) and black-pigmented bacilli were eliminated in 100% of the cases ($p<0.001$). Aerobic microorganisms were eliminated in 10 (83.3% reduction) ($p<0.001$) of the 12 cases positive before treatment, while streptococci were not found in 11 cases (64.7% reduction) ($p<0.001$). Mutans streptococci were eliminated in 5 (83.3% reduction) ($p<0.001$) of the 6 cases positive at first sampling. Gram-negative bacilli were not quantified.

After biomechanical preparation, the microorganisms were totally eliminated in 4 cases (20% reduction) ($p<0.001$), using thioglycolate medium (Figure 2).

After application of the intracanal calcium hydroxide dressing between sessions the anaerobic microorganisms were eliminated in 6 of the 7 cases positive after root canal mechanical preparation (85.7% reduction) ($p<0.001$). The remaining microorganisms were not quantified in any sample; thus, the dressing was efficient in eliminating quantified microorganisms (aerobic, streptococci and mutans streptococci) in 100% of the cases positive after root canal mechanical preparation ($p>0.001$) (Figure 1). In the thioglycolate medium, of the 16 samples positive after biomechanical preparation, microorganisms were eliminated in 10 samples (62.5% reduction) ($p<0.001$) after application of the between session intracanal dressing (Figure 2).

The cumulative action of root canal mechanical preparation and intracanal dressing eliminated the aerobic microorganisms, the streptococci and mutans streptococci in 100% of the cases ($p<0.001$) and also eliminated the anaerobic microorganisms in 95% of the cases ($p<0.001$) (Figure 1). This combined action of root canal mechanical preparation and intracanal dressing completely eliminated the microorganisms in 14 cases (70%) ($p<0.001$) in the thioglycolate medium (Figure 2).

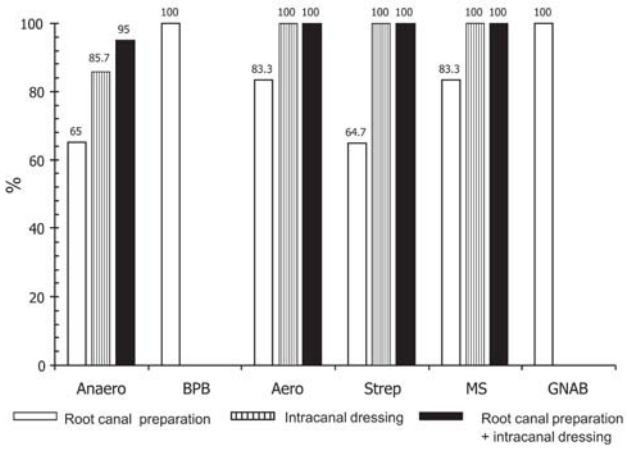

FIGURE 1- Efficiency (%) of the biomechanical preparation, intracanal dressing and the association of both on the elimination of anaerobic microorganisms (Anaero), black-pigmented bacillus (BPB); aerobic microorganisms (Aero), streptococci (Strep), mutans streptococci (MS) and gram-negative aerobic bacillus (GNAB)

DISCUSSION

The microbiological evaluations of the root canals were performed 72 h after root canal mechanical preparation and removal of the dressing as recommended by Leonardo et al.[7], who reported that bacteriological tests must be performed within the first 48/96 h after the endodontic intervention because the samples obtained immediately after antiseptic endodontic procedures do not reflect the real root canal system microbiological conditions.

In root canals of human primary teeth with pulp necrosis and apical periodontitis, the polymicrobial infection encountered had a predominance of anaerobic microorganisms. We observed that the root canal mechanical preparation led to a statistically significant reduction in the number of all evaluated microorganisms when compared to the initial counts (Figure 1). This was also true for the thioglycolate medium (Figure 2). However, this reduction was significantly greater with the combined action of root canal mechanical preparation and the intracanal dressing (Figures 1 and 2). These results are in accordance with Byström and Sundqvist[1]; Sjogren and Sundqvist[13] and Rodrigues and Biffi[10] who reported that in permanent teeth root canal mechanical preparation was not efficient in completely eliminating endodontic infection. Our results confirm that this is also true for primary teeth.

The maintenance of endodontic infection after root canal mechanical preparation probably occurred because in primary teeth with pulp necrosis and apical periodontitis as well as in permanent teeth, bacteria were not only present in the main canal but also frequently found in the dentinal tubules, forming biofilm in the external radicular surface at the apical region[5,8] which is inaccessible biomechanical preparation[7].

A real comparison of the decrease of the microbiota due to Calen paste with literature is impossible due to the lack of

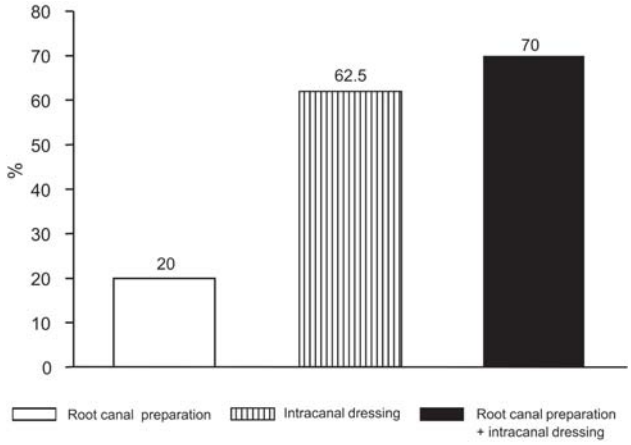

FIGURE 2- Efficiency (%) of the biomechanical preparation, intracanal dressing and the association of both on the elimination of microorganisms in the thioglycolate medium

in vivo studies evaluating the action of intracanal dressings in primary root canals.

The comparison between root canal mechanical preparation (20.0%) and the cumulative action of root canal mechanical preparation and intracanal dressing (70.0%) for the elimination of microorganisms from the root canal shows that the endodontic treatment of primary teeth with pulp necrosis and apical periodontitis cannot be performed in only one session. Therefore, our results indicate that the intracanal dressing is an important part of endodontic treatment in primary teeth with pulp necrosis and apical periodontitis.

CONCLUSIONS

Isolated root canal mechanical preparation showed inferior microbiological results (20%) when compared to its association with intracanal dressings (70%) indicating the necessity of topical application of an intracanal medication between sessions in primary teeth with pulp necrosis and apical periodontitis.

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