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ANTIMICROBIAL ACTIVITY OF SODIUM HYPOCHLORITE ASSOCIATED WITH INTRACANAL MEDICATION FOR *Candida albicans AND Enterococcus faecalis* INOCULATED IN ROOT CANALS

Marcia Carneiro VALERA[1], Katy Costa Godinho da SILVA[2], Lilian Eiko MAEKAWA[3], Cláudio Antonio Talge CARVALHO[4], Cristiane Yumi KOGA-ITO[5], Carlos Henrique Ribeiro CAMARGO[4], Raphael Silva e LIMA[2]

1- DDS, MSc, PhD, Adjunct Professor, Department of Restorative Dentistry, São José dos Campos Dental School, São Paulo State University, São José dos Campos, SP, Brazil.
2- DDS, Undergraduate student, Department of Restorative Dentistry, São José dos Campos Dental School, São Paulo State University, São José dos Campos, SP, Brazil.
3- DDS, MSc Graduate student, Department of Restorative Dentistry, São José dos Campos Dental School, São Paulo State University, São José dos Campos, SP, Brazil.
4- DDS, MSc, PhD, Assistant Professor, Department of Restorative Dentistry, São José dos Campos Dental School, São Paulo State University, São José dos Campos, SP, Brazil.
5- DDS, MSc, PhD, Adjunct Professor, Department of Oral Diagnosis and Biosciences, Department of Restorative Dentistry, São José dos Campos Dental School, São Paulo State University, São José dos Campos, SP, Brazil.

Corresponding address: Dra. Marcia Carneiro - Av. Engenheiro Francisco José Longo, 777 - Jd. São Dimas - 12245-000 - São José dos Campos, SP - Brasil.- Phone: +55-12-3947-9048 - e-mail: marcia@fosjc.unesp.br

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ABSTRACT

Objective: The purpose of this study was to evaluate the action of sodium hypochlorite (NaOCl) associated with an intracanal medication against *Candida albicans* and *Enterococcus faecalis* inoculated in root canals. Material and Methods: Thirty-six human single-rooted teeth with single root canals were used. The canals were contaminated with *C. albicans* and *E. faecalis* for 21 days and were then instrumented with 1% NaOCl. The roots were divided into 3 groups (n=12) according to the intracanal medication applied: calcium hydroxide paste, 2% chlorhexidine (CHX) gel, and 2% CHX gel associated with calcium hydroxide. The following collections were made from the root canals: a) initial sample (IS): 21 days after contamination (control), b) S1: after instrumentation, c) S2: 14 days after intracanal medication placement; S3: 7 days after intracanal medication removal. The results were analyzed statistically by the Kruskal-Wallis test at 5% significance level. Results and Conclusions: Both 1% NaOCl irrigation and the intracanal medications were effective in eliminating *E. faecalis* and *C. albicans* inoculated in root canals.

Key words: Sodium hypochlorite. Chlorhexidine. Calcium hydroxide. *Candida albicans*. *Enterococcus faecalis*.

INTRODUCTION

The microorganisms and their products are closely related to the etiology of pulpal and periapical lesions. They can cause pulp necrosis due to their persistence in the root canal system after endodontic treatment and can induce a periapical inflammatory reaction[13].

The polymicrobial nature of endodontic infections has been demonstrated. The root canal flora is formed by more than 700 different species and some of them have not yet been identified at species level. *Enterococcus faecalis*[15,23,29,34] has been frequently isolated in root canals with pulpal infection and also in secondary/persistent endodontic infections. In addition to bacteria, other microorganisms like yeasts can be found in root canals with pulp necrosis[18]. Yeasts are particularly associated with persistent root canal infections that do not respond favorably to conservative root canal therapy[31]. Najzar-Fleger, et al.[19] observed that 55% of root canals had yeasts of this type and Waltimo, et al.[30] verified for the polymerase chain reaction (PCR) analysis the presence of Candida spp. in root canals.

During biomechanical preparation, several chemical substances are used as irrigants. Due to a series of properties such as the capacity to dissolve the organic matter, lubrication and toxic content neutralization, sodium hypochlorite (NaOCl) is currently the most commonly used substance[3,24,33]. However, even after biomechanical preparation with antimicrobial substances, some microorganisms may survive in the root canal system, requiring disinfection by the use of an intracanal medication,

which will act beyond the root canal lumen, inside of dentinal tubules and apical resorptions[1,6,9,11].

Calcium hydroxide has been widely used as an intracanal medication due to its antimicrobial properties[1,17,25], especially due to its action over Gram-negative bacteria. However, it has a limited action against some microorganisms, particularly *E. faecalis*[9] and *Candida albicans*[2]. Estrela, et al.[8] verified that calcium hydroxide (CH) requires 60 days to have an antimicrobial effect on *C. albicans* and *E. faecalis* cultures. In failed root canal treatments, 2% chlorhexidine (CHX) gel may be a more effective intracanal medication than CH paste or their combination against *C. albicans* and *E. faecalis*[2]. *In vitro* studies have shown that when in direct contact, CH eliminated microorganisms present in the root canals[28]. However, *in vivo* studies have not reported similar performance in the root canal system[26,27].

The association of CH and 2% CHX has been used as intracanal dressing with encouraging results since it enhances the antimicrobial action of the paste against endodontic pathogens[6,10]. The purpose of this study was to evaluate the effect of biomechanical preparation with 1% NaOCl irrigation followed by intracanal medication with CH paste, 2% CHX gel and the association of these substances against *E. faecalis* and *C. albicans* inoculated in root canals.

MATERIAL AND METHODS

The present study was approved by the Research Ethics Committee of São José dos Campos Dental School, São Paulo State University, Brazil (Protocol #093/2005). Thirty-six freshly extracted human single-rooted teeth maintained in saline storage were used. The crowns were removed to provide a standardized root length of 16 ± 0.5 mm. The root canals were overinstrumented at 0.5 mm up to a size 25 K-file (Dentsply Ind. Com. Ltda, Petrópolis, RJ, Brazil), followed by instrumentation at 1 mm short of the apical foramen up to a size 30 K-file.

The external root surfaces were coated with epoxy resin (Araldite, Brascola, São Paulo, SP, Brazil), except for the coronal opening and apical foramen. The root canals were filled with 17% EDTA for 3 min and rinsed with 5 mL of saline. The apical foramen was then sealed with light-cured composite resin (Z-100; 3M ESPE, St. Paul, MN, USA) and the roots were autoclaved at 121°C for 15 min. Thereafter, the roots were joined to clear light-cured acrylic resin (Dencor; Artigos Odontológicos Clássico, São Paulo, SP, Brazil) in 24-well plates (Costar, Corning, NY, USA), which were sterilized by cobalt 60 gamma radiation (20 KGy for 6 h).

The microorganisms used were *C. albicans* (ATCC 18804) and *E. faecalis* (ATCC 29212). *C. albicans* was plated on Petri plates containing Sabouraud Dextrose agar (Himedia Laboratories, Mumbai, India) and incubated in a microbiological oven at 37 ± 1°C for 24 h. *E. faecalis* had been previously grown in Brain Heart Infusion (BHI) broth (Himedia Laboratories, Mumbai, India) for 48 h, and was then seeded on plates containing BHI agar BHI and incubated in a humidified incubator with 5% CO_2 at 37 ± 1°C for 48 h.

C. albicans and *E. faecalis* saline suspensions containing 10^8 cells/mL were prepared, corresponding to 1,258 optical density in the spectrometer at 530 nm wavelength (*C. albicans*) and 1,258 optical density in the spectrometer at 760 nm wavelength (*E. faecalis*). The root canals were contaminated with 5 mL of each microbial suspension plus 10 mL BHI and 10 mL Sabouraud broth, totalizing 30 mL inoculated in the root canal[17]. A sterile cotton ball was placed in the root canal opening and the coronal opening was sealed with a noneugenol, self-setting, single-component temporary coronal filling material (Coltosol; Coltene-Whaledent, Cuyahoga Falls, OH, USA). The plates were incubated in a microbiological oven at 37°C ± 1°C for 21 days and the culture medium (BHI broth) was added every 3 days to the root canals[17].

At the end of the contamination period, microbiological samples were collected to confirm contamination of root canals by the test microorganisms (control for each group). After control collection, the root canals were instrumented up to a size 50 K-file with 5 mL of 1% NaOCl at each change of instrument and 3 groups were formed (n=12) according to the intracanal medication: CH paste (Calen® paste; SS White, Rio de Janeiro, RJ, Brazil; composition: 2.5 g CH, 0.5 g zinc oxide, 0.05 g colophony and 1.75 mL polyethylene glycol 400 (vehicle)); 2% CHX gel (Byoformula Drugstore, São José dos Campos, SP, Brazil); 2% CHX gel associated with CH powder (Inodon; Porto Alegre, RS, Brazil), at 1:1 mixing ratio[11].

The following collections were made from the root canal: a) initial sample (IS): 21 days after contamination (control), b) S1: after instrumentation with 1% NaOCl irrigation, c) S2: 14 days after placement of the intracanal medication; S3: 7 days after removal of the medication. Between S2 and S3, the canals were filled with sterile saline.

Before the first collection of microbiological samples (IS), the roots were irrigated with 0.06% sodium thiosulfate for NaOCl neutralization. All collections from root canals, namely immediately after instrumentation (S1), 14 days after intracanal medication (S2), and after 7 days after removal of the medication and filling with saline (S3), were made in the same way. A sterile size 50 paper point was left in the root canal for 1 min. After this time, each paper point was placed in an Eppendorf test tube containing 0.5 mL of sterile saline, shaken for 30 s, and 0.1 mL of the microbial suspension were double seeded on plates containing culture medium for *C. albicans* and *E. faecalis*. After growth check, the formation of *E. faecalis* and *C. albicans* colonies was confirmed by Gram staining under light microscopy. Data were analyzed statistically by the Kruskal-Wallis test at a significance level of 5%.

RESULTS

The mean values of colony forming units (cfu)/mL obtained in the groups after confirmation collection (SI -

TABLE 1- Mean values of cfu/mL obtained in the groups after confirmation collection (SI - control), 1st collection (biomechanical preparation – S1), 2nd collection (14 days after placement of the intracanal medication – S2) and 3rd collection (7 days after removal of the of intracanal medication)

Group	Confirmation collection (IS-control)		1st collection (S1)		2nd collection (S2)		3rd collection (S3)	
	C. albicans	*E. faecalis*	*C. albicans*	*E. faecalis*	*C. albicans*	*E. faecalis*	*C. albicans*	*E. faecalis*
CH paste	1414.22	272.08	0.208	1.708	0	0	2.66	0
2% CHX gel	2441.25	342	0.166	0.25	0.083	17.50	0.166	0.125
CH + 2% CHX gel	1455.16	377.66	0	0	0	0	0	0

IS = initial sample; CH = calcium hydroxide; CHX = chlorhexidine; cfu= colony forming units

control), 1st collection (biomechanical preparation – S1), 2nd collection (14 days after placement of the intracanal medication – S2) and 3rd collection (7 days after removal of the of intracanal medication).are presented in Table 1.

Statistically significant difference ($p<0.05$) was found between the IS and S1. Considering that after placement of the intracanal medication the mean cfu/mL values of both *C. albicans* and *E. faecalis* were the same or close to zero, there was no need to apply the statistical test to the results.

No statistically significant differences ($p>0.05$) were found between the intracanal medications against *C. albicans* and *E. faecalis*.

DISCUSSION

Biomechanical preparation with NaOCl has been proven effective in eliminating microorganisms present in the root canal system. Even though the antimicrobial action of NaOCI has not yet been fully understood, hypochlorite acid is formed in the presence of water containing active chlorine, a powerful oxidizing agent that produces an antimicrobial effect by irreversible oxidation of hydrosulphuric groups of essential enzymes, disturbing the metabolic functions of the bacterial cell. Chlorine can also adhere to bacterial cytoplasm components forming highly toxic N-chloro composites that destroy the microorganisms[24].

Byström and Sundqvist[4] reported that 0.5% NaOCI was more effective than saline as an irrigant, confirming the antimicrobial properties of that substance. Although less concentrated solutions have shown antimicrobial effectiveness[24], higher concentrations of NaOCI present faster and greater bactericidal effect[10,22]. However, the higher the concentration of this substance, the greater its cytotoxic effect[10,22]. In the present study, 1% NaOCl was effective to considerably reduce *C. albicans* and *E. faecalis* counts immediately after root canal preparation.

Nevertheless, NaOCl has limited capacity to penetrate into the dentinal tubules. Vahdaty, et al.[32] observed the antimicrobial action of NaOCI against E. *faecalis* up to 500-µm deep, while Sen, et al.[21] reported that, even when 5.25% NaOCI was used, it was impossible to eliminate C. *albicans* from human dentin sections after 1-h exposure. Berutti, et al.[3] observed an increase in the antibacterial effect of 5.0% NaOCI when used alternately with 10% EDTA solution. This is related to the demineralizing action of EDTA, which prevents smear layer formation during instrumentation, resulting in an increased NaOCI penetration into the dentinal tubules.

In the present study, the groups dressed with calcium hydroxide paste and 2% CHX gel exhibited a considerable decrease in the number of cfu/mL after biomechanical preparation with NaOCI irrigation (S1), while the group dressed with 2% CHX gel plus calcium hydroxide presented total elimination of microorganisms at S1. It must be considered that no microorganisms were expected to be found after that collection. It is likely that if the same canals had been filled with saline and sealed for 7 days they would have shown contamination due to migration of microorganisms that had remained in the dental tubules and branches of the root canal system, as reported elsewhere[17]. However, the purpose of this study was to evaluate the biomechanical preparation using an irrigant with antimicrobial activity followed by placement of intracanal medication simulating the clinical conditions. The use of an intracanal medication could reduce or even eliminate the microorganisms inoculated in the root canal, as demonstrated by the empty canals at 7 days after removal of medication.

Sodium thiosulfate 0.06% and tween 80% + lecithin 0.07% are well known substances in neutralizing the residual activity of NaOCl and CHX. However, an adequate CH neutralizer solution has not been found. Therefore, in order to standardize the experimental conditions of the present study, no neutralizer was used after 14 days of intracanal medication.

The 2% CHX gel showed optimal antimicrobial action against *E. faecalis* and *C. albicans*, resulting in microbiological collections close to zero or minimum growth (S2). Then, it was noted that after removal of the intracanal medication, the number of cfu/mL of *E. faecalis* was higher than after S3. It is likely that the action of the intracanal medication during 14 days was not sufficient to eliminate *E. faecalis*. However, the residual effect of 2% CHX gel completed the action initiated when the medication was in

the root canal. Even so, complete elimination of microorganisms was not observed. Previous studies using 2% CHX gel as a chemical auxiliary substance to biomechanical preparation showed its effectiveness against microorganisms present in the root canal[5,12,14]. The CHX acts by electrostatic interaction, that is, it is positively charged and the bacterial wall is negatively charged. This interaction increases the cell wall coating, allowing bacterial cytoplasm coagulation, resulting in cell death[7].

In this study, it was noted that the association of CH and CHX as intracanal medication was able to keep the root canal free from microorganisms that could possibly remain inside the dentinal tubules after biomechanical preparation. The mechanism of action of this association is the same as the CH and CHX. The CH, associated or not with other substances, is the most used medication as root canal dressing in Endodontics[24,33]. Its antimicrobial activity is primarily related to the release of hydroxyl ions with consequent pH increase, as well as the action of hydroxyl ions on the inactivation of enzymes of the cytoplasmic membrane of bacteria, which influences the chemical transportation and alteration in the availability of nutrients, causing toxic effects to the bacterial cells[25]. McHugh, et al.[16] reported that the pH between 10.5 and 11 delays the growth of *E. faecalis* and that only a pH higher than 11 can eliminate this microorganism. In the present study, the CH paste completely eliminated the microorganisms at the 2nd collection (S2) and permitted only minimum growth of *C. albicans* at the 3rd collection (S3). These results show the effectiveness of this medication against *E. faecalis* and *C. albicans*. The association of CH and CHX combines the antimicrobial properties of CHX against other microorganisms not tested in the present study[7,11] with the mineralization potential and neutralization properties against endotoxins of CH[20].

CONCLUSION

Biomechanical preparation with 1% NaOCl irrigation considerably reduced *C. albicans* and *E. faecalis* counts in contaminated root canals. The tested intracanal medications were also able to produce a significant decrease in the number of microorganisms, so that the association of CH and CHX maintained the root canals completely free from microorganisms.

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