Antibacterial action of intracanal medicaments on infected dentin of deciduous and permanent teeth

Giovanna P. S. R. **REZENDE**[1]
Daniel A. **DECURCIO**[1]
Carlos **ESTRELA**[2]
Luciane R. R. S. **COSTA**[3]

ABSTRACT

Objective: The purpose of this study was to evaluate the antimicrobial effectiveness of intracanal medicaments on infected dentin of deciduous and permanent teeth. **Methods:** Dentin blocks were inoculated with *Enterococcus faecalis* every 72 h for 60 days; then they were irrigated, dried and completely filled with one of the following mixtures: 1) Calcium hydroxide powder, propolis and propylene glycol; 2) calcium hydroxide powder and propylene glycol; 3) calcium hydroxide powder and sterilized water; 4) propolis and propylene glycol; 5) propolis and sterilized water. After 30 days, the samples were washed with sterilized water, immersed in Letheen Broth and incubated for 48 hours at 37 °C. **Results and Conclusion:** The hypothesis that the association of calcium hydroxide with propolis would be more effective than the medicaments themselves was not confirmed, as the results indicated that all the mixtures tested were not able to inhibit *E. faecalis* biofilm, either in dentin blocks of deciduous or permanent teeth.

Keywords: Calcium hydroxide. Propolis. Biofilms. *Enterococcus faecalis*.

How to cite this article: Rezende GPSR, Decurcio DA, Estrela C, Costa LRRS. Antibacterial action of intracanal medicaments on infected dentin of deciduous and permanent teeth. Dental Press Endod. 2011 July-Sept;1(2):34-9.

» The authors report no commercial, proprietary, or financial interest in the products or companies described in this article.

Received: December 10, 2010. Accepted: January 1, 2011.

[1]Postgraduate Student of Health Sciences, Federal University of Goiás, Goiânia, GO, Brazil.
[2]Professor of Endodontics, Federal University of Goiás, Goiânia, Brazil.
[3]Associate Professor of Pediatric Dentistry, Federal University of Goiás, Goiânia, GO, Brazil.

Contact address: Luciane R. R. S. Costa
School of Dentistry, Federal University of Goiás 1ª Avenida s/n, Setor Universitário, Goiânia GO, Brazil, 74.605-220 – E-mail: lsucasas@odonto.ufg.br

Introduction

It is well known that endodontic infections have a polymicrobial nature either in deciduous[1] or permanent teeth,[2] being *Enterococcus faecalis* one important microorganism to be controlled. *E. faecalis* was observed by polymerase chain reaction in 22% and 32% of necrotic deciduous and permanent root canals, respectively,[3] and it is more prevalent in secondary than in primary endodontic infections.[4] *E. faecalis* is a nonspore-forming, fermentative, facultative anaerobic, and gram-positive coccus that can colonize the dentinal walls from the root canals, adhering to the mineral part as well as to the collagen through different virulence factors.[4,5]

Several substances have been tested in attempt to eliminate *E. faecalis* from root canals, aiming an optimal outcome in non-vital pulp therapy. Although calcium hydroxide has been widely used in endodontics due to its various biological properties,[6] it cannot eliminate *E. faecalis* when used as a solely agent in infected dentin models.[7] A recent systematic review concluded that calcium hydroxide is little effective against bacteria from human root canal.[8] Antimicrobials such erythromycin and oxytetracycline, beside calcium hydroxide, were effective in erradicating two-day-old *E. faecalis* biofilm, whereas ampicillin, co-trimoxazole, and vancomycin followed by gentamicin were ineffective.[9]

Propolis might be a satisfactory adjunct substance for pulp therapies in deciduous and permanent teeth. Propolis is a resinous substance collected by bees that has been extensively used for centuries as a natural chemical agent against infectious diseases.[10] Particularly in endodontics, propolis has been suggested for root canal desinfection, so reducing and controlling pulp and periapical inflammatory reactions, inducing the healing process and controlling post-treatment pain and discomfort.[11] As an endodontic antimicrobial agent, a 10% ethanol extract of propolis is quite effective against *Prevotella nigrescens*, presents intermediate action against *Fusobacterium nucleatum*, but shows high minimum inhibitory and bactericidal concentrations against *Actinomyces israelii* and *E. faecalis*, using the broth macrodilution method.[12] Similarly *E. faecalis* was the most resistant Gram-positive bacterium in regards to 20% ethanol extract of propolis,[13] but propolis solutions have demonstrated good activity against *E. faecalis* in single-rooted canal after short-term application in two experiments using infected model from human permanent teeth.[14,15]

Even though it was reported that experimental pastes containing calcium hydroxide and 11% propolis extract were effective against polymicrobial cultures collected from necrotic root canals in deciduous molars by means of the agar-well diffusion method,[16] little is known about the antimicrobial properties of this mixture. This study aimed to assess the effectiveness of the association 'calcium hydroxide and propolis' against *E. faecalis* in an infected dentin model, to test the hypothesis that this combination might be a satisfactory intracanal medication for endodontic infections in deciduous and permanent teeth.

Materials and methods

Test microorganism

A bacterial strain obtained from the American Type Culture Collection (*Enterococcus faecalis*, ATCC 29212) was inoculated into 7 ml of Brain Heart Infusion (BHI; Difco, USA) and incubated at 37 ºC for 24 hours. The experimental suspension was prepared on the surface of Brain Heart Infusion agar; bacterial cells were resuspended in saline to be adjusted to tube 1 of the MacFarland scale (3×10^8 cells/ml).

Teeth preparation

A total of 42 root samples were prepared from deciduous and permanent extracted maxillary first molars. The deciduous teeth had at least two thirds of root length and absence of perforation in the root wall or furcation area; and the permanent had complete rhyzogenesis, without resorptions. This study was approved by Research Ethics Committee at the Federal University of Goiás, Brazil (protocol number 62/2006). Teeth were obtained from dentists and from a teeth bank, after the donors (adults and children's legal guardian) had consented for the use of their teeth in this research.

Experimental procedures

All teeth were decoronated at the cement-enamel junction. Then blocks of 4 mm and 5 mm height for deciduous and permanent teeth, respectively, were removed from the cervical palatal root with a diamond disk #7020 (KG Sorensen, São Paulo, Brazil) at low speed and under water cooling. The blocks had their cementum layer removed by a cylindrical diamond bur #3101 (KG Sorensen) in a high-speed handpiece and under water cooling.

Each dentin block was individually shaped, using K-files #15 to #30 for deciduous teeth and, K-files #15 to #40 (Maillefer®, Switzerland) for permanent teeth; then they were enlarged with Gates-Glidden drills #2 (deciduous)

and #3 (permanent). Dentin blocks were continuously irrigated with 1% sodium hypochlorite (Miyako®, Guarulhos, SP, Brazil) during the mechanical preparation.

Samples were separated into 7 groups for deciduous and 7 for permanent teeth, each one containing three root dentin blocks in accordance with the medicaments associations specified in the first two columns of Table 1. Dentin blocks were dried and filled with 17% EDTA (pH 7.2) for 3 min; after cleaning and shaping they were sterilized by autoclaving (30 min at 120 °C).

The design of the dentin infection was based on previous studies.[17,18] All blocks (except the negative control) were initially inoculated with *E. faecalis* strains. This procedure was repeated every 72 h during 60 days, always using 24 h cultures adjusted to tube 1 of the MacFarland turbidity standard. Blocks were maintained in a humid environment at 37 °C.

After 60 days, positive control was used to check bacterial viability throughout the experiment. Subsequently, the other blocks were irrigated with 5 ml of saline solution, dried with two sterile gauze and four sterilized absorbent paper points, and completely filled with the intracanal medicament. After this, they were immediately placed in Petri dishes containing 1 g of the medicament (sufficient to cover the blocks) and maintained during 30 days. Subsequently, the samples were individually washed with 10 ml of sterilized water, transported and immersed in a respective tube with 7 ml of Letheen Broth (Difco, USA), homogenized and incubated for 48 hours at 37 °C. Microbial growth was analyzed by turbidity of the culture medium. Afterward, an inoculum of 0.1 ml obtained from Letheen Broth was transferred to 7 ml of BHI under identical incubation conditions. All assays were carried out in duplicate. Each block was scored as either positive or negative for viable *E. faecalis* under the growth conditions.

Results

The associations of propolis with calcium hydroxide, as well as other associations of propolis or calcium hydroxide with other vehicles were not able to inhibit *E. faecalis* growth on dentin blocks extracted from deciduous or permanent teeth (Table 1). Bacteria were viable in the positive control group, while the negative control group was free of microorganisms.

Table 1. Atimicrobial activity of studied medications on *E. faecalis* biofilm.

Group	Association of medicaments	Dentin blocks[a]	
		Deciduous teeth	Permanent teeth
1	0.4 g calcium hydroxide powder[b] 0.1 g pure, dry extract of propolis[c] 0.2 ml propylene glycol[d]	+++	+++
2	0.5 g calcium hydroxide powder[b] 0.2 ml propylene glycol[d]	+++	+++
3	0.5 g calcium hydroxide powder[b] 0.2 ml sterilized water	+++	+++
4	0.5 g pure, dry extract of propolis[c] 0.2 ml propylene glycol[d]	+++	+++
5	0.5 g pure, dry extract of propolis[c] 0.2 ml sterilized water	+++	+++
	Positive control group	+++	+++
	Negative control group	- - -	- - -

[a] Symbol represents bacterial growth in repeated experiments: (+++) indicates positive growth result i.e. presence of *E. faecalis* and medication inneficacy for each dentin block; (- - -) represents negative result (absence of growth or medication efficacy) for each dentin block. [b] Biodinâmica, Ibiporã, PR, Brazil. [c] Pool of Brazilian propolis (patent pending, Apis Flora, Ribeirão Preto, SP, Brazil). [d] Henrifarma, São Paulo, SP, Brazil.

Discussion

This study in infected human dentin demonstrated no antimicrobial effectiveness of the association 'propolis and calcium hydroxide' against *E. faecalis* biofilm. Within the conditions of this study the same result occurred to calcium hydroxide with propylene glycol or with sterilized water and to propolis with propylene glycol or with sterilized water. The positive cultures of *E. faecalis* following the application of all medicaments suggest their inability to disinfect the human root canal, confirming that the reduction of cell viability inside biofilms is extremely difficult. The complex internal anatomy of root canals offers opportunity of microorganisms surviving in the inaccessible and remote areas promoting a good environment for growth, multiplication, and interaction in pulp infections,[19,20] despite it refers to deciduous or permanent teeth.

The method of dentin blocks used in this study attempted to reproduce a clinical situation even though the microorganisms were obtained *ex vivo* from pure culture collection. Dentin was contaminated with *E. faecalis* for 60 days, allowing bacteria penetration into the dentinal tubules to form a biofilm. Clinically, the level of bacterial invasion into the root dentinal tubules is also related to the incubation period: The extended exposure of the root canal to the oral environment results in significant amplified microbial invasion of the root canal system.[21]

Other studies have assessed the antimicrobial activity of intracanal medicaments using infected dentin blocks removed from different sources: human teeth *in vivo*[14,15,18] and *ex vivo,*[17,22] and dog's teeth *in vivo*.[23,24] However, none of them had investigated dentin samples from deciduous teeth. Considering the lower permeability of deciduous molars,[25] one could expect that bacteria would be able to invade easier and deeply the dentin tubules of permanent teeth becoming more aggressive and difficult to eliminate, but this was not the case in the present study. Probably the contamination period was enough to allow the infection of deciduous molars' dentin.

It should be emphasized that the results could be different if we had investigated the same medicaments on planktonic cells as it happened with another antimicrobial agent: Ozone had an antibacterial effect on planktonic *E. faecalis* and on those suspended in fluid, but little effect was observed when this microorganism was embedded in biofilms.[18] Moreover, both propolis and calcium hydroxide as antibacterial agents were effective against *E. faecalis* only in macrodilution method.[12] Multiple resistance mechanisms such as the production of an exopolysaccharide protective matrix and the modulation of the gene expression pattern are related to an increased bacterial resistance in biofilms: Biofilm bacteria can become up to 1,000 times stronger against an antimicrobial than their planktonic counterparts.[26]

In this study, the association of medicaments aimed to produce an intracanal medication able to eliminate *E. faecalis*. However, the antimicrobial agents were applied after the microorganism inoculation without any previous irrigation. Considering that irrigants are important to optimize intracanal disinfection others results may be waited if we had used an effective irrigant solution.[27] Chemicals that alter the physicochemical properties of dentin can influence the nature of adherence, adhesion force, and subsequent *E. faecalis* biofilm formation on dentin.[28]

It is difficult to contrast the results of different studies on propolis antimicrobial activity against *E. faecalis* due to differences in propolis formulations as well as in microbiologic methods. The results of the present study are in disagreement with other two investigations that concluded that propolis is effective against *E. faecalis* biofilms.[14,15] Both of them used shorter contamination periods, e.g. 7 days[14] and 21 days.[15] In addition, one tested a 10% ethanolic extract of bursa propolis[14] and the other, a 30% solution of Jordanian propolis.[15] The contamination period of *E. faecalis* in dentin was discussed earlier in this section. As the composition of propolis varies according to the region where propolis is collected,[10] one could speculate that Brazilian propolis would not be so effective. However, previous studies using other microbiologic methods have reported that *E. faecalis* was susceptible to Brazilian propolis.[12]

Nevertheless, the findings in the present study are in agreement with those investigations that reported that calcium hydroxide medications are not able to eradicate *E. faecalis* biofilm[27]. This ineffectiveness is due to the poor diffusion of hydroxyl ions into infected dentin and the buffering capacity of dentin, reducing its alcalinization potential.[29] Moreover, the dentinal collagen can confer resistance to the bacterium against calcium hydroxide.[30]

To sum up, calcium hydroxide and propolis were not able to eliminate 60 day *E. faecalis* biofilm *in vitro*. Considering that *in vitro* assessments do not exactly

reproduce clinical outcomes, further studies using different methods should answer the questions that have arisen. Nevertheless, calcium hydroxide plays an important role in endodontics as long as vital pulp therapies are concerned. Propolis should be further tested under other experimental conditions though. One cannot ignore the broad properties of propolis and its potential application in endodontics as a pulpotomy agent in deep carious lesions, as intracanal medications in primary endodontic infections of permanent teeth, and as filling agent in deciduous teeth pulpectomies.

Acknowledgments

This study was partially supported by a grant from Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), Brazil.

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