Received: 21st September 2014 Accepted: 28th December 2014 Conflicts of Interest: None

Source of Support: Nil

Original Research

Antimicrobial Activity of Filling Materials Used in Primary Teeth Pulpotomy

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How to cite the article:

Pimenta HC, Borges AH, Bandeca MC, Neves AT, Fontes RG, da Silva PV, Aranha AM. Antimicrobial activity of filling materials used in primary teeth pulpotomy. J Int Oral Health 2015;7(4):54-57.

Abstract:

The aim of this study was to investigate the antibacterial activity of pulp capping materials used in primary teeth (formocresol [FC], zinc oxide and eugenol cement [ZOE], ZOE mixed with FC [ZOEFC], mineral trioxide aggregate [MTA] and calcium hydroxide [CH]) against cariogenic bacteria. The agar plate diffusion test was used for the cultures, including saline solution as a negative control. A base layer of 15 mL of brain heart infusion agar was inoculated with 300 mL of each inoculum. Twelve wells were made and completely filled with one of the testing materials for each bacteria strain. The plates were incubated at 37°C for 48 h. Zones of microbial inhibition and material diffusion were measured and photographed. The results obtained were analyzed by Kruskal–Wallis and Mann–Whitney non-parametric tests. Respectively, the medium zones of bacteria inhibition of FC, ZOE, ZOEFC, MTA and CH against *Streptococcus mutans* growth were 28.5, 15.2, 20.8, 9.3 and 11.6; against *Lactobacillus acidophilus* growth were 28.7, 14.8, 15.3, 15.2 and 20.0, and against *Actinomyces viscosus* growth were 13.6, 13.5, 14.7, 10.0 and 13.6. We might confirmed the high antibacterial activity of FC solution, especially against *S. mutans* and *L. acidophilus,* as wells as, the low inhibitory effect of MTA cement on the cariogenic bacteria studied.

Key Words: Anti-infective agents, deciduous, pulpotomy

Introduction

One of the major purposes of pediatric dentistry is to maintain primary teeth in anatomical and functional conditions up to their physiological exfoliation and eruption of permanent teeth. It is fundamental to prevent changes in chewing, speech and phonetics, as well as for maintenance of the dental arch length and esthetics, and prevention of deleterious oral habits.[1]

The early childhood caries as in dental traumas may involve pulp tissue, exposing it to oral microflora.[2] In the absence of endodontic treatment and bacterial control, inflammatory mediators are released and can compromise the vitality of dental pulp, leading the tissue to dystrophic calcification or to necrosis.[3,4]

Currently, there are different techniques and protocols for the treatment of the pulp of primary teeth, depending on the damage extension and the pathologic pulp involvement.[5] Pulpotomy is a conservative therapeutic alternative responsible for the removal of the infected coronal pulp and capping of the remaining non-infected pulp tissue with biocompatible material.[4,5] The ideal pulp capping cement should have good physical and biological properties such as sealing of the remaining pulp tissue, not being resorbable, being biocompatible and presenting antibacterial activity.[3] An antibacterial pulp capping cement is necessary to avoid failures in the conservative endodontic treatment and the dissemination of the inflammatory and infectious processes along the radicular channel.[3]

Among the materials used for primary teeth pulpotomies stand out the calcium hydroxide (CH) cement.[3,6] and formocresol (FC).[7-10] The CH cement has the ability to stimulate the tertiary dentin formation, it is biocompatible and presents high alkaline pH, which confers it antimicrobial property.[3,6,11] However, when not correctly indicated, it may stimulate internal dentinal resorption.[12] FC is a medication derived from formaldehyde, widely accepted as the treatment of choice for primary teeth,[7] characterized by the clinical action of tissue fixation, but it does not lead to tissue repair.[10] Although there are evidences of clinical success and radiographic success,[8] there is controversy about FC toxicity and carcinogenic potential,[7,13] which explain its decreasing use in recent years.[9] In this regard, techniques and more biocompatible alternative materials have been proposed, including laser therapy, lyophilized bone, bone morphogenetic protein and aggregate of mineral trioxide.[14]

The MTA has attracted the attention of endodontics as a result of its sealing capacity, biocompatibility, ability to stimulate hard tissue deposition and periodontal ligament regeneration, and cell inductive potential.[14,15] MTA is composed mainly of calcium phosphate and calcium oxide, with an initial pH of 10.2 and during its setting reaches a pH of 12.5, comparable to CH.[3]

In addition, there is evidence that MTA presents antimicrobial properties similar to zinc oxide and eugenol cement (ZOE) and low cytotoxic effect.[16] Clinically and radiographically, MTA has been showed a 100 % success rates after 6, 12 and 24 months treatment.[12,17]

As the microorganisms eradication is essential for successful endodontic treatment, characterized by the maintenance of the vitality of the remaining radicular pulp, the evaluation of the antimicrobial activity of filling materials used in pulpotomy of primary teeth is critical to the success of the technique.

Materials and Methods

The materials evaluated in this study were FC (FC; Biodynamic Chemistry and Pharmaceutical Ltda., Ibiporã, PR, Brazil), ZOE (ZOE; IRM, Dentsply, Petrópolis, PR, Brazil), FC mixed with ZOE (FCZOE), mineral trioxide aggregate (MTA; Ângelus, Londrina, PR, Brazil), CH paste (CH p.a and physiological solution) (HC; Biodynamic Chemistry and Pharmaceutical Ltda., Ibiporã, PR, Brazil). The antimicrobial activity of each paste was evaluated against *Streptococcus mutans* (ATCC # 25175), *Lactobacillus acidophilus* (ATCC# IAL-523) and *Actinomyces viscosus* (T14V # IAL.5) by the Agar diffusion test. Indicators strains were grown in Brain Heart Infusion Agar (BHI™ - Difco Laboratories, Detroit, MI, USA) at 37°C for 24 h, according to the physiological characteristics of each microorganism.

In each sterilized petri plates (20 mm × 100 mm), a base layer containing 15 mL of BHI agar mixed with 300 mL of each inoculum was prepared. After solidification of culture medium, six wells measuring 4 mm in diameter, about 15 mm from the edges of the dishes and at equidistant points, were made in each plate and completely filled with one of the testing materials. For the FC group, 5 mL of FC were applied on sterile filter paper discs ($N = 12$). Twelve wells were filled with each material and bacterial strain. All materials were prepared under aseptic conditions according to the recommendations of their respective manufacturers. 5 mL of 0.9% saline solution was applied on a 4 mm diameter sterile filter paper discs ($N = 3$), as negative control group. The plates were kept for 2 h at room temperature for diffusion of the material. After that, they were incubated at 37°C for 48 h.

Zones of bacterial inhibition and material diffusion were photographed and measured in millimeters (mm), using a digital caliper (Mitutoyo, São Paulo, SP, Brazil). Measurements were taken at the greatest distance between two points at the outer limit of the inhibition halo formed around the well. Antibacterial tests were repeated three times to confirm the homogeneity of the results. Results were expressed as means and subjected to statistical non-parametric tests of Kruskal–Wallis and Mann–Whitney (Wilcoxon rank-sum-tests) at the significance level of 5%.

Results

The mean values and standard deviations of the inhibition zones for each material according to the bacteria strain are shown in Table 1. Antibacterial activity was only considered to have occurred when a true inhibition zone was present, whether associated or not with the diffusion zone.

It can be observed that all studied material showed antibacterial activity, and they were statistically different from the negative control against bacteria studied strains. FC was the most effective material against all tested bacteria strains. When associated to the FC, the ZOE increased their activity against *S. mutans*. MTA showed the lowest activity against *S. mutans* and *A. viscosus.*

Only the CH paste showed diffusion zones between 3.8 and 5.7 mm for all bacteria strains.

Discussion

In this study, the antimicrobial activity of FC, CH paste, ZOE associated or not with FC, and MTA cement were investigated. FC and CH paste have been used worldwide as pulp capping materials in primary teeth pulpotomy[10,18-20] and more recently, MTA, due to showing good clinical and radiographic results and being biocompatible.[12,14,17]

The microorganisms studied in this study are Gram-positive aerobic bacteria strains, which are part of the aciduric phase of the dental caries progression.[21] Dental caries is resulted by imbalance of the de-/remineralization process where strains of *S. mutans* and *Lactobacillus* as well as aciduric strains of non-mutans *Streptococcus*, *Actinomyces*, bifidobacteria (anaerobic Gram-positive bacteria) and fungi may be dominant.[21] Around 30-50% of the microorganisms present in the caries lesions are represented by *S. mutans,* but including *L. acidophilus* and *A. viscosus* presence.[22]

Different in vitro methods have been used to study antibacterial activity of dental materials.[23,24] Although differences in the agar medium, capacity of diffusion of the materials, bacterial strains,

Table 1: Means of inhibition zones diameters (mm) and SD values observed on inoculated agar plates after 48 h according to experimental material and bacteria strain.

Material (SD)	Bacteria		
	S. mutans	*L. acidophilus*	*A. viscosus*
FC	28.5±7.26^{a*}	28.7±3.25^{a}	13.6±3.58^{b}
ZOE	15.2±0.56^{b}	14.8±1.22^{b}	13.5±0.93^{b}
FCZOE	20.8±4.28^{a}	15.3±2.15^{b}	14.7±1.1^{b}
MTA	9.3±0.54^{c}	15.2±1.19^{b}	10.0±2.88^{c}
HC	11.6±0.41^{c}	20.0±0.66^{a}	13.6±0.60^{b}
SS	0±0.0^{d}	0±0.0^{d}	0±0.0^{d}

*Means followed by the same letters are not different according to the Mann–Whitney test ($P>0.05$). FC: Formocresol, ZOE: Zinc oxide and eugenol cement, FCZOE: Formocresol mixed to zinc oxide and eugenol cement, MTA: Mineral trioxide aggregate, HC: Calcium hydroxide paste; SS: 0.9% saline solution, SD: Standard deviation, *S. mutans: Streptococcus mutans, L. acidophilus: Lactobacillus acidophilus, A. viscosus: Actinomyces viscosus*

and cell density may interfere in the formation of inhibition zones around the materials studied,[11] the agar plate diffusion test was selected in this study to be the method of antimicrobial activity widely used.[23,25,26]

According to the results of the present study, FC was the most effective medication against *S. mutans* and *L. acidophilus,* having high activity against *A. viscosus* as well. FC has been considered the gold standard in pediatric dentistry due to excellent clinical and radiographic results in pulpotomy of primary molars.[10,18] CH paste presented best effect against *L. acidophilus* followed by *A. viscosus*. It could be explained by its high alkalinity, reaching a pH of 12.5 after setting,[27] and the fact that both bacteria strains are highly acidogenic and aciduric, and may survive in an environment at pH less than 4.6.[28] However, the action mechanism of CH to bacteria is not yet totally clarified.[29]

MTA presented less antimicrobial activity against *A. viscosus* and notably against *S. mutans* than the other materials. The MTA setting process is based on the reactions of anhydrous cement compounds with water (hydration reaction), producing a calcium silicate hydrate of low alkalinity, which dissociates in CH molecules.[30] Thus, MTA, after setting, may be considered as CH surrounded by a silicate matrix.[30] Although presented inferior results in vitro test, MTA has shown clinical and radiographic results compatible to FC,[10,12,17,18] and higher than CH.[12,19] In addition, MTA presents other ideal properties as its high sealing ability, biocompatibility, capacity to stimulate dentin neoformation and regeneration of periodontal tissue.[12,17]

Conclusions

The present study demonstrated that all tested materials indicated for pulpotomy of primary teeth have antibacterial potential, according to bacteria strain. More evidences on the mechanisms of action of released components by materials, pH, as well as on the strains metabolism, are necessary to explain the results obtained. Another important issue to be investigated is whether the antimicrobial activity is sufficient to control bacterial metabolism or eliminate these microorganisms.

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