Antimicrobial Analysis of Different Root Canal Filling Pastes Used in Pediatric Dentistry by Two Experimental Methods

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The objective of this study was to compare, by two experimental methods, the antimicrobial efficacy of different root canal filling pastes used in pediatric dentistry. The tested materials were: Guedes-Pinto paste (GPP), zinc oxide-eugenol paste (OZEP), calcium hydroxide paste (CHP), chloramphenicol + tetracycline + zinc oxide and eugenol paste (CTZP) and Vitapex®. Fiven microbial strains (*S. aureus*, *E. faecalis*, *P. aeruginosa*, *B. subtilis* and *C. albicans*) obtained from the American Type Culture Collection were inoculated in Brain Heart Infusion (BHI) and incubated at 37ºC for 24 h. For the direct exposure test (DET), 72 paper points were contaminated with the standard microbial suspensions and exposed to the root canal filling pastes for 1, 24, 48 and 72 h. The points were immersed in Letheen Broth (LB), followed by incubation at 37ºC for 48 h. An inoculum of 0.1 mL obtained from LB was then transferred to 7 mL of BHI, under identical incubations conditions and the microbial growth was evaluated. The pastes showed activity between 1 and 24 h, depending on the material. For the agar diffusion test (ADT), 30 Petri plates with 20 mL of BHI agar were inoculated with 0.1 mL of the microbial suspension, using sterile swabs that were spread on the medium. Three cavities were made in each agar plate (total = 90) and completely filled with one of the filling root canal pastes. The plates were pre-incubated for 1 h at room temperature and then incubated at 37ºC for 24 to 48 h. The inhibition zone around each well was recorded in mm. The complete antimicrobial effect in the direct exposure test was observed after 24 h on all microbial indicators. All root canal filling materials induced the formation of inhibition zones, except for Vitapex® (range, 6.0-39.0 mm).

Key Words: calcium hydroxide, intracanal dressing, pediatric dentistry.

INTRODUCTION

The success of endodontic treatment in pediatric dentistry is directly influenced by the elimination of the microorganisms in infected root canals. The individual species of the endodontic microbiota are usually of low virulence, but collectively they are pathogenic due to a combination of factors. Apical periodontitis is stimulated by microbial toxins, enzymes and their byproducts. Special conditions facilitate the maintenance of polymicrobial infections, as the selective pressures related to oxidation-reduction potential, nutrient supply, microbial interactions, and less natural defense (1).

Different intracanal medicaments have been purposed for use in pediatric dentistry (1-20). Viable bacteria can be recovered from the root canals after being treated by an effective disinfection process. Therefore, especially in pediatric dentistry, difficulties in antimicrobial control require the use of root canal filling pastes with broad antimicrobial activity.

Benfatti and Andrioni (2) tested the bacteriostatic effect of zinc oxide-eugenol plus Formocresol, Ox-

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para, zinc oxide and eugenol containing Lincomycin and zinc oxide and eugenol against *Streptococcus* and *Staphylococcus*. All drugs had a marked inhibition of bacterial growth. The largest halo of inhibition was produced by the drug Ox-para.

Tchaou et al. (3) compared the antibacterial effectiveness of 10 materials: 1) calcium hydroxide mixed with camphorated parachlorophenol (CH + CPC); 2) calcium hydroxide mixed with sterile water (CH + H_2O); 3) zinc oxide mixed with CPC (ZnO + CPC); 4) zinc oxide mixed with eugenol (ZOE); 5) ZOE mixed with formocresol (ZOE + FC); 6) zinc oxide mixed with sterile water (ZnO + H_2O); 7) ZOE mixed with chlorhexidine dihydrochloride (ZOE + CHX); 8) Kri paste; 9) Vitapex® paste; 10) vaseline (control). These materials were compared against microbial species obtained from 13 infected primary teeth by using an agar diffusion test. The results suggested that the materials could be divided into 3 categories. Category I, with the strongest antibacterial effect, included ZnO + CPC, CH + CPC and ZOE + FC; category II, with an intermediate antibacterial effect, included ZnO + CHX, Kri, ZOE, and ZnO + H_2O; category III, with no or minimal antibacterial effect, included Vitapex®, CH + H_2O and vaseline. There were no significant differences within each category, but there were significant differences among the categories. The only exception was the antibacterial effect of ZOE + FC, which was not significantly different from ZOE + CHX, Kri or ZOE.

Bonow et al. (4) evaluated the immediate and residual activity of several pastes (Guedes-Pinto paste, zinc oxide, calcium hydroxide and mixed pastes) and liquids (formocresol, 1/5 formocresol and 2% glutaraldehyde) used during pulp therapy of primary teeth on pure microbial culture by agar diffusion test. Zinc oxide paste had the strongest and 1/5 formocresol the weakest antimicrobial activity. There was statistically significant difference among all materials, except for 2% glutaraldehyde and formocresol that were similar to each other.

Pabla et al. (5) evaluated the antimicrobial efficacy of various root canal filling materials for primary teeth. Four materials (zinc oxide and eugenol, iodoform paste, Kri paste, Maisto paste and Vitapex®) were tested against aerobic and anaerobic bacteria (*S. aureus*, *S. viridans*, *S. faecalis*, *B. melaninogenicus* and mixed bacterial culture) obtained from infected non-vital primary anterior teeth. The antimicrobial sensitivity was checked on BHI-agar plates using the well method. All materials were statistically different from each other regarding their antimicrobial activity. Maisto paste had the best antibacterial activity. Iodoform paste was the second best followed by zinc oxide and eugenol and Kri paste. Vitapex® showed the least antibacterial activity.

Different results have been found in several studies (2-11). The experimental model to evaluate antimicrobial action of endodontic materials used in pediatric dentistry should be considered. The purpose of this study was to compare, by two experimental methods, the antimicrobial efficacy of different root canal filling pastes used in pediatric dentistry.

MATERIAL AND METHODS

Microbial Indicators. Five standard microbial strains obtained from the American Type Culture Collection were used in this study: *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633) and one yeast, *Candida albicans* (ATCC 10231).

Medicaments. Five root canal filling pastes were used: *Guedes-Pinto paste* (GPP) [composed of 0.30 g iodoform (K-Dent; Quimidrol, Joinville, SC, Brazil), 0.25 g Rifocort (Merrel Lepetit, Santo Amaro, SP, Brazil) and 0.1 mL camphorated paramonochlorophenol]; *Zinc oxide and eugenol paste* (ZOEP) (SS White, Rio de Janeiro, RJ, Brazil); *Calcium hydroxide paste* (CHP), prepared with calcium hydroxide p.a. (Quimis Mallinkrodt, Inc.; St. Louis, MO, USA) and propyleneglycol (Natu Phamas, Goiânia, GO, Brazil); *Chloramphenicol + tetracycline chloride + Zinc oxide and eugenol paste* (CTZP) (Neo Química, Anápolis, GO, Brazil + Cifarma, Santa Luiza, MG, Brazil + SS White); Vitapex® (DiaDent Group International Inc., Burnaly, BC, Canada). The pastes were prepared with a toothpaste consistence.

Direct Exposure Test (DET). The strains were inoculated in 7 mL of Brain Heart Infusion (BHI; Difco Laboratories, Detroit, MI, USA) and incubated at 37°C for 24 h. The 5 biological indicators were cultivated on Brain Heart Infusion Agar (BHIA; Difco Laboratories) following the same incubation conditions. Microbial cells were re-suspended in saline to give a final concentration of about 3×10^8 cells/mL, similar to that of tube #1 of MacFarland scale. One mL of each pure suspensions was used to obtain a mixture of the test organisms.

Seventy-two sterile #50 absorbent paper points (Tanari, Tanariman Indústria, Ltda., Manacaru, AM, Brazil) were immersed for 5 min in the experimental suspension prepared with the mixture of the 5 microorganisms, and were then placed on Petri plates and covered with one of the 5 root canal filling pastes, or with saline (control group). At 1-, 24-, 48- and 72-h intervals, 18 absorbent paper points were removed from contact with the medicaments, individually transported and immersed in 7 mL of Letheen Broth (LB; Difco Laboratories), a medium containing neutralizers and recommended for inhibition tests, followed by incubation at 37°C for 48 h. Microbial growth was analyzed by turbidity of the culture medium. Subsequently, an inoculum of 0.1 mL obtained from LB was transferred to 7 mL of BHI, under identical incubation conditions. Bacterial growth was also evaluated by culture medium turbidity. Gram stain of BHI cultures was used to asses the contamination and growth was determined under macroscopic and microscopic (Gram stain) examinations. All assays were carried out in triplicate under aseptic conditions.

Agar Diffusion Test (ADT). Thirty Petri plates with 20 mL of BHI agar were inoculated with 0.1 mL of the microbial suspensions (*S. aureus* + *E. faecalis* + *P. aeruginosa* + *B. subtilis* + *C. albicans* + mixture), using sterile swabs that were spread on the medium, obtaining growth in junction. Three cavities (4 mm in depth and 4 mm in diameter) were made in each agar plate (total = 90) with a copper coil and completely filled with the root canal filling pastes. The plates were pre-incubated for 1 h at room temperature, and then incubated at 37°C for 24-48 h. Microbial inhibition diameters were measured. Positive and negative controls were done, keeping the plates inoculated and without inoculum, for the same periods and under identical incubation conditions. All assays were carried out under aseptic conditions.

RESULTS

The results of the direct exposure test and agar diffusion test are shown on Tables 1 and 2, respectively. The complete antimicrobial effect in the direct exposure test was observed after 24 h on all microbial indicators. All root canal filling materials induced inhibition zones, except for Vitapex® (range, 6.0-39.0 mm).

Table 1. Antimicrobial efficacy of root canal filling pastes on mixed microbial cultures. Results of each repeat experiment.

Pastes	1 h	24 h	48 h	72 h
GPP	+++	- - -	- - -	- - -
ZOEP	- - -	- - -	- - -	- - -
CHP	+++	- - -	- - -	- - -
CTZP	- - -	- - -	- - -	- - -
Vitapex®	+++	- - -	- - -	- - -
Saline	+++	+++	+++	+++

+++ (Positive result) = growth presence/inefficacy; - - - (Negative result) = absence of growth/efficacy. GPP = Guedes-Pinto paste; ZOEP= zinc oxide and eugenol paste; CHP = calcium hydroxide + propyleneglycol paste; CTZP = chloramphenicol + tetracycline + zinc oxide and eugenol paste).

Table 2. Means of the diameters (in mm) of the inhibition zones in the agar diffusion test.

Pastes	Microorganisms					
	S. aureus AGPC	*E. faecalis* AGPC	*P. aeruginosa* AGNR	*B. subtillis* AGPR	*C. albicans*	Mixture
GPP	12	8	26	13	32	10
ZOEP	12	15	9	16	12	10
CHP	9	11	10	9	8	6
CTZP	39	8	39	37	33	13
Vitapex®	0	0	0	0	0	0

AGPC = aerobic Gram-positive coccus; AGNR = aerobic Gram-negative rod; AGPR = aerobic Gram-positive rods, spore-forming. GPP = Guedes-Pinto paste; ZOEP= zinc oxide and eugenol paste; CHP = calcium hydroxide + propyleneglycol paste; CTZP = chloramphenicol + tetracycline + zinc oxide and eugenol paste).

DISCUSSION

The main goal of root canal therapy in pediatric dentistry is the maintenance of primary teeth until the correct eruption of the permanent dentition, under healthy conditions. Several materials have been proposed for the treatment of endodontic infections in primary teeth (2-11).

Considering the particularity of primary teeth, the complete sanitization process requires the application the root canal filling materials with properties such as antimicrobial potential and biocompatibility. Therefore, some of the pastes most commonly used by Brazilian pediatric dentists were tested in the present study - Guedes-Pinto paste, zinc oxide and eugenol paste, calcium hydroxide paste, CTZ paste and Vitapex®. The use of two methods was based on the findings of a previous work (12) that showed different results depending on the experimental test.

The results indicate that, by the direct exposure test, all root canal filling pates showed antimicrobial efficacy against *S. aureus*, *E. faecalis*, *P. aeruginosa*, *B. subtilis* and *C. albicans*. The results obtained by agar diffusion test showed that the CTZ paste presented the strongest antimicrobial activity, followed by Guedes-Pinto paste, zinc oxide and eugenol paste and calcium hydroxide paste. The Vitapex® showed the worst results.

Establishing the spectrum of activity of any antimicrobial agent is useful for improvement of infection control. In general, three *in vitro* techniques have been used for such purpose: the dilution method, which yields a quantitative result for the amount of antimicrobial agent that is required; the agar diffusion method, which gives an inhibition zone around the wells containing the agent; and the direct exposure method, which provides qualitative information about the substance. All techniques have advantages and disadvantages. The dilution method can only be used with substances that are soluble in the culture medium. The size of the microbial inhibition zone depends on the solubility and diffusibility of the test substance in the agar diffusion method and, therefore, may not express its full potential. The direct exposure method is correlated to substance effectiveness and its direct contact with microorganisms. This method is independent on other variables and is practical to be used in the laboratory (1).

The study design should also be considered. Estrela et al. (12) investigated two methods for determining the antimicrobial efficacy of calcium hydroxide plus saline, calcium hydroxide plus propyleneglycol, and calcium hydroxide plus camphorated paramonochlorophenol. Those authors (12) reported that both the direct exposure test and the agar diffusion test are useful to establish the antimicrobial spectrum of calcium hydroxide, thus, improving infection control protocols. Complete antimicrobial efficacy was observed after 48 h for 6 microorganisms, by both tests, irrespective of the vehicle associated with the calcium hydroxide.

The agar diffusion test, which evaluates halos of microbial growth inhibition as reference parameters, may not offer equal conditions to compare substances with different solubility and diffusibility and the correct performance of microbiological technique. Factors such as pre-incubation, dried culture medium and maintenance for periods that exceed the ideal time for analysis can yield doubtful results (1,12,13). The agar diffusion test does not distinguish between bacteriostatic and bactericidal properties of dental materials neither does it provide any information about the viability of the test microorganism, nor its limitation to measure the activity of soluble components. The agar diffusion test requires careful standardization of inoculum density, medium content, agar viscosity, size and number of specimens *per* plate (1,12-15).

In the present study, CTZP and Guedes-Pinto pastes had the best results in the agar diffusion test. However, the size of the microbial inhibition zone depends upon the solubility and diffusibility of the test substance, which means that the results of a material may not express its full potential. Guedes-Pinto et al. (6) evaluated clinically and radiographically the efficacy of Guedes-Pinto paste on 45 pulp necrotic teeth with furca (bi or tri) involvement, having or not dental fistula. The teeth were examined in the following periods: 1 week; 1, 3 and 6 months; 1 and 2 years after treatment. This technique showed to be highly successful (97.8%). On the other hand, Mani et al. (7) evaluated calcium hydroxide and zinc oxide-eugenol as root canal filling materials in primary teeth. The teeth were evaluated every 2 months for a period of 6 months, using various clinical and radiographic criteria. The high success rate (86.7%) and the absence of any adverse response in the present study have proved the favorable properties of CH. This positive finding and its nonfixative nature are sufficient to warrant its use as a root canal filling material in primary teeth. Costa et al. (8) compared the response of subcutaneous tissue to an antibiotic cement (tetracycline associated with chloramphenicol) to that produced by zinc oxide and eugenol cement. Poliethylene tubes filled with the cements were implanted in dorsal skin subcutaneous surgical pockets of 25 rats. The animals were sacrificed 3, 7, 15 30 and 60 days after these procedures. Serial sections of the skin stained with HE showed that the antibiotic cement was less irritant than the ZOE cement only in 3 initial periods.

Estrela et al. (1), analyzing the control of microorganisms by calcium hydroxide pastes, reported that an antimicrobial effect occurs after 48 h on *S. mutans, E. faecalis, S. aureus, P. aeruginosa, B. subtilis, C. albicans* and a mixed suspension containing all microorganisms, regardless of the vehicle (saline, camphorated paramonochlorophenol, 1% chlorhexidine solution, 3% sodium lauryl sulfate, corticosteroid-antibiotic). The lack of differences within the time required for antimicrobial effect by direct contact indicates that these vehicles play a supportive role in the process, giving the pastes chemical characteristics such as dissociation and diffusion, as well as enhancing the filling of root canals, which are decisive factors for antimicrobial potential and tissue healing ability.

In the present study, the Vitapex® showed the worst results. However, Nurko and Garcia-Godoy (9) studied the effectiveness of Vitapex® (calcium hydroxide/iodoform paste) in the root canal treatment of primary teeth. The treatment was deemed successful if, clinically, the tooth was painless, without pathological mobility, and the gingival was healthy and without a fistula. The authors observed that the main advantages of the Vitapex® paste are that its resorbs from the apical tissues within 1 week to 2 months, is apparently harmless to permanent tooth germs, is radiopaque, does not set to a hard mass and is easily inserted/removed. On the other hand, Estrela et al. (16) verified the influence of iodoform on the antimicrobial potential of calcium hydroxide on *S. aureus*, *E. faecalis*, *P. aeruginosa*, *B. subtilis and C. albicans* by direct exposure test and agar diffusion test. The results showed significant antimicrobial effectiveness for calcium hydroxide paste associated with saline or iodoform plus saline.

Murata (17) analyzed histomorphologically three root canal filling materials (Maisto paste, Sealer 26 with iodoform, L&C paste - calcium hydroxide with oliver oil) in 80 root canals of dogs' primary anterior teeth from 6 sixty-day old animals. Thirty days after the treatment, the animals were sacrificed and the pieces prepared for histomorphological analysis. The statistical analysis ordered the materials from the best to the worse results in the following way: a) Maisto paste; b) Sealer 26 with iodoform; c) control group; d) L&C. L&C paste was not biologically compatible and the other materials were biocompatible but its resorption was not in the same rate as that of the roots of the primary teeth.

Analyzing the two experimental methods in this study, the performance of calcium hydroxide paste was considered as good. Calcium hydroxide has shown expressive antimicrobial superiority during the treatment of infected root canals in comparison to camphorated paramonochlorophenol (and with camphorated phenol) using bacteriological methods (18). Holland et al. (19) reported that the addition of camphorated paramonochlorophenol to calcium hydroxide (Calen® + CMCP; SS White) did not improve the treatment outcomes, comparing to tissue healing process 6 months after root canal filling in dogs' teeth with periapical lesions. Estrela and Holland (20) stablished important parameters on calcium hydroxide's properties based on scientific evidences. They concluded that calcium hydroxide's characteristics come from its dissociation into calcium and hydroxyl ions. The action of these ions on tissues and bacteria explains the biological and antimicrobial properties of this substance. Under the conditions of this discussion, it is possible to state that: 1. Dentin is considered the best pulpal protective and calcium hydroxide has proved its capability of inducing the formation of a mineralized bridge over pulpal tissue; 2. It is necessary, whenever possible, to provide time for calcium hydroxide paste to manifest its potential of action against the microorganisms present in endodontic infections. The maintenance of a high concentration of hydroxyl ions can change bacteria enzymatic activity and promote its inactivation; 3. The site of action of hydroxyl ions of calcium hydroxide includes the enzymes in the cytoplasmic membrane. This medication has a large scope of action and is therefore effective against a wide range of microorganisms, regardless their metabolic capability. Microbial cytoplasmic membranes are similar, irrespective of microorganisms' morphological, tinctorial and respiratory characteristics, which means that this medication has a similar effect on aerobic, anaerobic, Gram-positive and Gram-negative bacteria. 4. The use of alcium hydroxide as a temporary interappointment root canal dressing promotes better results on the periapical healing process than the treatment in one appointment.

Further research is required to determine the antimicrobial efficacy of other root canal filling pastes for use in infected root canals in pediatric dentistry.

Based on the results of this study and within the limitations of this methodology, the following conclusions may be drawn: the direct exposure test revealed that all root canal filling pastes (GPP, OZEP, CHP, CTZP, Vitapex®) had an overall antimicrobial effect against all microbial indicators after 24 h. In the agar diffusium test, all materials induced the formation of inhibition zones, except for Vitapex® (range, 6.0-39.0 mm).

RESUMO

O objetivo do presente estudo foi comparar o efeito antimicrobiano de diferentes pastas obturadoras do canal radicular usadas na Odontopediatria, por dois métodos experimentais. Os materiais testados foram: pasta Guedes Pinto, pasta de óxido de zinco e eugenol, pasta de hidróxido de cálcio, pasta CTZ e Vitapex®, sobre cinco microrganismos (*S. aureus*, *E. faecalis*, *P. aeruginosa*, *B. subtilis* e *C. albicans*) obtidos do *American Type Culture Collection*. As cepas foram inoculadas no *Brain Heart Infusion* (BHI) e incubadas a 37ºC por 24 h. Para o teste de contato direto, 72 pontas de papel foram contaminadas com suspensões padrão dos microrganismos e expostas às pastas obturadoras por 1, 24, 48 e 72 h. As pontas foram imersas em *Letheen Broth* (LB), seguido de incubação a 37ºC por 48 h. Um inóculo de 0,1 mL obtido do LB foi transferido para 7 mL de BHI, sobre condições idênticas de incubação e o crescimento microbiano foi avaliado. As pastas mostraram ação entre 1 e 24 h, dependendo da pasta testada. Para o teste de difusão em ágar, 30 placas de Petri com 20 mL de agar BHI foi inoculada com 0,1 mL da suspensão microbiana, utilizando-se de *swab* esterilizado, semeado de modo confluente no meio. Três cavidades foram feitas em cada placa de ágar (total = 90) e completamente preenchidas com uma das pastas obturadoras. As placas foram pré-incubadas por 1 h em temperatura ambiente e então incubadas a 37ºC por 48 h. As zonas de inibição em torno das cavidades foram mensuradas. O efeito antimicrobiano completo obtido pelas pastas analisadas, por meio do teste por contato direto, foi observado após 24 h em todos os microrganismos. No teste por difusão em ágar, todos os materiais induziram a formação de zonas de inibição, exceto o Vitapex® (variando de 6,0 – 39,0 mm).

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Accepted May 27, 2006