Antimicrobial action of root canal filling pastes used in deciduous teeth

Ação antimicrobiana de pastas obturadoras usadas em dentes decíduos

Abstract

Purpose: This study assessed the antimicrobial effectiveness of an iodoform-based paste (Guedes-Pinto Paste, GPP) and a paste modified by the addition of a 2% chlorhexidine gluconate gel (CHX) to replace the camphorated parachlorophenol component of the original GPP.

Methods: The antimicrobial action was tested against the following microorganisms: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus oralis*, *Enterococcus faecalis*, *Escherichia coli* and *Bacillus subtilis*. In the agar diffusion test, 18 Petri plates with 20 mL of BHIA were inoculated with 0.1 mL of the microbial suspensions. Paper disks were immersed in the experimental solutions for 1 min and were then placed over the BHIA surface in each agar plate. The plates were maintained for 1 h at room temperature and then incubated at 37°C for 48 h. The diameters of the microbial inhibition halos were measured around the paper disks containing the substances. Data were analyzed using the Mann-Whitney U-test ($\alpha=0.05$).

Results: Antimicrobial action was observed for the GPP and CHX pastes, which presented absence of turbidity for almost all microorganisms. No statistically significant difference in the antimicrobial action was found between GPP and CHX.

Conclusion: Both pastes present similar antimicrobial effectiveness against several microorganisms commonly found in endodontic infections in deciduous teeth.

Key words: Pulpectomy; deciduous teeth; chlorhexidine

Resumo

Objetivo: Este estudo avaliou a ação antimicrobiana de pastas, uma à base de iodofórmio conhecida por Pasta Guedes-Pinto (PGP) e outra modificada pela adição de digluconato de clorexidina (CHX) a 2% em substituição ao paramonoclorofenol canforado da formulação original da PGP.

Metodologia: A ação antimicrobiana das duas pastas foi testada contra *Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus oralis, Enterococcus faecalis, Escherichia coli* e *Bacillus subtilis*. O método empregado foi o de Diluição em Meio Sólido, Difusão em Ágar. Dezoito placas de Petri com 20 mL de BHIA foram inoculados com 0,1 mL das suspensões microbianas. Discos de papel foram imersos nas soluções experimentais por 1 min e colocados sobre a superfície de BHIA em cada placa. As placas foram mantidas em temperatura ambiente por 1 h e então incubadas a 37°C por 48 h. O diâmetro da inibição microbiana foi medida ao redor dos discos de papel. Os dados foram pelo teste U de Mann-Whitney ($\alpha=0,05$).

Resultados: A PGP teve ação bacteriostática contra todos os microrganismos e também bactericida exceto para *Enterococcus faecalis* e *Bacillus subtilis*. A CHX apresentou ação bacteriostática e bactericida contra todos os microrganismos. Não houve diferença estatística significante quanto à efetividade antimicrobiana entre as pastas avaliadas.

Conclusão: Ambas as pastas apresentaram ação antimicrobiana contra quase todos os microrganismos encontrados em infecções endodônticas de dentes decíduos.

Palavras-chave: Pulpectomia; dentes decíduos; clorexidina

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Introduction

Pulp therapy in deciduous teeth is a complex treatment due to the instrumentation procedures, the complexity of the apical delta (1-3), the biological cycle of the deciduous teeth (1-2), physiological root resorption and rhizolysis (1,3-4) and long treatment sessions during which the child sometimes does not always cooperate (5-6). Therefore, it is important to select an effective material for endodontic treatment of deciduous teeth to eliminate microorganisms from the root canal system using an appropriate set of procedures, such as biomechanical preparation of the root canal, irrigation with different solutions and the use of intracanal dressings. An ideal root canal filling material for deciduous teeth must have several properties, such as being harmless to the periapical tissues and permanent teeth germs, resorbing readily if pressed beyond the apex, and being antiseptic (3-6). Then, the success of the endodontic therapy is primarily dependent on the susceptibility of the infecting microorganism to the commonly used antimicrobial agents (1,2).

For pulpectomy cases in deciduous teeth, over 90% of all dental schools in Brazil have adopted the root filling paste proposed by Guedes-Pinto et al. (7), which is called Guedes-Pinto Paste (GPP) and is composed of iodoform, camphorated parachlorophenol (CMPC) and Rifocort® (5-9). The successful combination of these three drugs in the endodontic therapy of deciduous teeth has been reported by several studies (2,4,5-7,10-13), showing excellent biocompatibility with pulp fibroblasts and mild inflammatory reactions. These three drugs have also been shown to be well tolerated by periapical and connective tissues (3). Santos (14) assessed the *in vitro* cytotoxicity of the Guedes-Pinto paste in comparison with formocresol, glutaraldehyde and phosphoric acid. The NIH 3T3 cell line (ATCC CRL 1658) and pulp fibroblasts (FP1) were used for the cell viability tests, and the paste was directly applied to coverslips placed in contact with the cells in a short-term assay (cell viability) up to 12 h and in a long-term assay (cell survival) from 1 to 7 days. In that study, the group treated with GPP had more viable cells than the group treated with the other drugs; GPP cytotoxicity was reduced in fibroblast culture tests, and CMPC was the most cytotoxic c omponent (14).

No studies have attempted to modify GPP by removing CMPC and adding another substance with antimicrobial activity and low cytotoxicity. Chlorhexidine has been used in periodontology for more than 20 years due to its antimicrobial properties and low cytotoxicity (1,15-21). It also can be used as an endodontic irrigation solution (15-18,20) and as an intracanal medication (15,19-20).

Therefore, the aim of this *in vitro* study was to evaluate the antimicrobial effectiveness of an iodoform-based paste (Guedes-Pinto Paste-GPP) and a paste modified by the addition of a 2% chlorhexidine gluconate gel (CHX) to replace the CMPC component of the original GPP against microorganisms commonly found in endodontic infections of deciduous teeth.

Methodology

The two filling pastes were prepared by mixing equal parts of each component on a sterilized glass plate, as follows:

1) GPP – 0.25 g Rifocort®; 0.30 g iodoform and 0.1 mL CMPC (3:7).
2) CHX – 0.25 g Rifocort®; 0.30 g iodoform and 0.05 mL 2% chlorhexidine digluconate.

The antimicrobial action of the tested pastes was investigated using a microbial mixture composed of the following microorganisms: *Staphylococcus aureus* (ATCC29213), *Staphylococcus epidermidis* (ATCC12228), *Streptococcus oralis* (ATCC10557), *Enterococcus faecalis* (ATCC29212), *Escherichia coli* (ATCC25922) and *Bacillus subtilis* (ATCC6633). All of the strains, which had been isolated and identified by the American Type Culture Collection (ATCC), were cultivated and maintained at the Department of Microbiology of the Federal University of Santa Maria, Santa Maria, RS, Brazil.

The strains were used to inoculate 7 mL of brain heart infusion (BHI; Difco Laboratories, Detroit, MI, USA), which was then incubated at 37º C for 24 h. The six biological indicators were cultivated on the surface of brain heart infusion agar (BHI; Difco Laboratories) following the same incubation conditions. Microbial cells were resuspended in saline to give a final concentration of $3x10^8$ cells/mL, similar to that of tube #1 of the MacFarland scale. One milliliter of each of these pure suspensions was used to obtain a mixture of the test microorganisms.

For the agar diffusion test, 18 Petri plates containing 20 mL of BHIA were inoculated with 0.1 mL of the microbial suspensions using sterile swabs that were spread on the medium. Fifty-four paper disks (9 mm in diameter) were immersed in the experimental solutions for 1 min, and then three paper disks were placed over the BHIA surface in each agar plate. The plates were maintained for 1 h at room temperature and were then incubated at 37ºC for 48 h. The diameter of the microbial inhibition halo was measured in millimeters around the paper disks containing the substances. Microbial growth was evaluated by the turbidity of the culture medium. Gram staining of the BHI cultures was used identify contamination, and growth was determined with macroscopic and microscopic (Gram stain) examination. All assays were carried out in duplicate under aseptic conditions.

The Mann-Whitney U-test was used to compare the diameters of the microbial inhibition halos of the tested pastes at the 5% level of significance.

Results

Antimicrobial action was observed for the GPP and CHX pastes, which presented absence of turbidity for almost all microorganisms. GPP proved to be bacteriostatic for *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus oralis*, *Enterococcus faecalis*, *Escherichia coli* and *Bacillus subtilis* and was bactericidal for all

microorganisms except for *Enterococcus faecalis* and *Bacillus subtilis*. The CHX paste showed bacteriostatic and bactericidal action for all microorganisms.

There was no significant difference in antimicrobial effectiveness between the pastes ($P>0.05$) (Table 1).

Table 1. Descriptive statistics of the antimicrobial effectiveness (inhibition halo – in mm) of the GPP and CHX pastes

	Mean (IC 95%) *	Median	Min	Max	Std. Dev.
GPP	8.51 (5.00-16.50)	7.00	4.00	20.00	4.40
CHX	6.13 (1.25-14.00)	5.00	1.00	14.00	4.20

* No significant difference between groups (Mann-Whitney U-test, $\alpha=0.05$).

Discussion

The success of endodontic treatment depends on several factors, but primarily on the reduction or elimination of bacterial infection (21). Microorganisms and their by-products are considered the major cause of pulp and periradicular pathologies. Hence, a major objective in root canal treatment is to disinfect the entire root canal system, which requires that all of its content is eliminated as a possible source of infection. This goal may be accomplished using mechanical instrumentation and chemical irrigation in conjunction with medication of the root canal system between treatment sessions (22-23). Therefore, it is necessary to pair an appropriate technique with a low cytotoxic material, which should have excellent antimicrobial properties against the microorganisms usually found in contaminated canals (5-6). Chemical substances used in pulp therapy in deciduous teeth should be well tolerated, should be able to be resorbed physiologically (21), must have excellent antiseptic properties associated with the ability to diffuse into the dentinal tubules (23) and should maintain the tooth in a functional state until its normal exfoliation, without endangering the permanent dentition (3).

In the present study, both pastes showed antimicrobial effectiveness against almost all microorganisms commonly found in endodontic infections of the root canal system. GPP is the most widely used material used in pulpectomy of deciduous teeth in Brazil. Kramer et al. (9) found that 48% used GPP as a root canal filling, followed by zinc oxide and eugenol paste (ZOE) (19%), ZOE with iodoform (7%) and GPP with ZOE (7%). Other studies (2,4,5-7,9-13) also reported that GPP had microbiological, histopathological and low cytotoxicity aspects that make it suitable to be used as a root filling material for deciduous teeth. GPP has antimicrobial action related to its components (7): iodoform has a germicidal effect (8,13), CMPC is an antimicrobial agent with an effective bacteriostatic action when administered either topically or systematically (10) but is cytotoxic (14), and Rifocort® is a combination of a corticosteroid and an antibiotic that has a great antimicrobial action and is recommended for the treatment of deciduous teeth with endodontic infections (3,5-6). Santos (14) showed that CMPC was the component responsible for the cytotoxicity in GPP, although the level of cytotoxicity was low.

The present study was the first to test CHX as a replacement for CMPC in GPP, and we showed that the CHX paste had antimicrobial action against all microorganisms. Chlorhexidine gluconate has been used in endodontics as an irrigation solution (15-18,20) and as an intracanal medication (15,17,19). It is active against a wide range of microorganisms, such as Gram-positive and Gram-negative bacteria (1,15-21). In addition to its low cytotoxicity and high antimicrobial activity, CHX has other properties that make its use suitable in endodontic treatments, such as the ability to interact with oral tissues (1,15,17,20-21) and to continue to be released for a long period of time (17,20-21). CHX gel is an effective intracanal medication due to its broad antimicrobial spectrum (1,15-21), which is in agreement with the present findings. This study showed that the 2% CHX gel produced inhibition zones and was effective against all microorganisms. Thus, chlorhexidine should be used as intracanal medication due to its safety and antimicrobial properties (1).

The agar diffusion method used in the present study is commonly employed to evaluate antimicrobial activity (2,4,5,6,10,13,23). According to Estrela et al. (15), the inhibition halo size depends on the solubility and diffusion of the substance; therefore, the antimicrobial potentials of drugs with different consistencies, such as a paste and a liquid, cannot be compared directly. Tanomaru et al. (21) showed that the contact between the tested material and the agar; the weight, size and molecular shape of the antimicrobial agent; the loading and concentration of the tested material; the agar gel texture; and the ionic concentration of the medium should be considered when using this method. In addition to these aspects, this method has been widely used to test antimicrobial activity (2,4,5,6,10,13,22), which is relevant to pulp therapy practice.

GPP has been proven to be bacteriostatic for *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus oralis, Enterococcus faecalis, Escherichia coli* and *Bacillus subtilis* and to be bactericidal for all of these microorganisms except *Enterococcus faecalis* and *Bacillus subtilis*. CHX paste showed bacteriostatic and bactericidal action for all microorganisms. These microorganisms were selected because they are commonly found in infected root canals of deciduous teeth (5,6,10,13,17). Additionally, some of the microorganisms served as references in quality control procedures for antimicrobial sensitivity tests, such as *Enterococcus faecalis*, *Escherichia coli* and *Staphylococcus aureus* (20,24-25). *Enterococcus faecalis* was the most resistant organism with respect to the antimicrobial action, as previously shown (4,5,6,15,20). *Enterococcus faecalis* can be found in the oral cavity flora through contamination and is part of the microbiota of infected canals of deciduous teeth (4), invading the pulp and dental tubules in cases of pulpitis and necrosis (4). *Bacillus subtilis* does not participate in the

oral pathogenic flora and was only included in this study as a quality parameter for the sensitivity test of the antimicrobial agents (4,5).

The interpretation of these results should be cautious to avoid direct extrapolations of *in vitro* findings to the clinics. The results of this paper suggest that the antimicrobial activity and cytotoxicity of 2% chlorhexidine digluconate when used for the endodontic treatment of deciduous teeth should be assessed. Further studies are required to identify the alternative material that has the largest number of desirable characteristics for use as a root canal filling for deciduous teeth with endodontic infection.

Conclusions

In accordance with the methodology used and the results obtained, it can be concluded that the replacement of CMPC by CHX in GPP did not negatively affect the antimicrobial effectiveness of the root canal filling paste.

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