

Exploiting the antibacterial effects of nine formulations of intracanal medication and irrigants against several bacterial strains

Explorando os efeitos antibacterianos de nove formulações de medicação intracanal e irrigantes contra várias cepas bacterianas

Exploración de los efectos antibacterianos de nueve formulaciones de medicamentos intracanal y irrigantes contra varias cepas bacterianas

Received: 05/05/2022 | Reviewed: 05/30/2022 | Accept: 06/11/2022 | Published: 06/20/2022

Thais Machado de Carvalho Coutinho

ORCID: <https://orcid.org/0000-0003-1266-9679>

Iguaçu University, Brazil

E-mail: thaiscoutinhoendo@gmail.com

Andrea Fagundes Campello

ORCID: <https://orcid.org/0000-0003-2328-5021>

Iguaçu University, Brazil

Grande Rio University, Brazil

E-mail: andreamcampello@hotmail.com

Sileno Correa Brum

ORCID: <https://orcid.org/0000-0003-4659-3422>

Iguaçu University, Brazil

E-mail: brumsc@uol.com.br

Carlos Henrique Sardenberg Pereira

ORCID: <https://orcid.org/0000-0002-8746-5681>

Iguaçu University, Brazil

E-mail: carlossardenberg@gmail.com

José Claudio Provenzano

ORCID: <https://orcid.org/0000-0003-4902-8664>

Iguaçu University, Brazil

E-mail: odontojcp@gmail.com

Flávio Rodrigues Ferreira Alves

ORCID: <https://orcid.org/0000-0002-9922-8202>

Iguaçu University, Brazil

Grande Rio University, Brazil

E-mail: flavioferreiraalves@gmail.com

Abstract

Elimination of intraradicular infection is essential for the success of endodontic treatment. This study evaluated the antibacterial effects of nine different formulations of intracanal medication and irrigating solutions, based on calcium hydroxide (Ca(OH)₂), calcium sulfate (CaSO₄), and a chlorhexidine (CHX), against nine bacterial strains. Aerobic or facultative anaerobic bacteria were grown in trypticase-soy broth and the anaerobic strain was grown in pre-reduced anaerobically sterilized brain heart infusion. The evaluation was performed by using the agar diffusion test in aerobic and anaerobic conditions using plates containing mitis-salivarius agar or trypticase-soy agar. The results showed that practically all substances tested had some antibacterial activity, except for calcium hydroxide and calcium sulfate when associated only with glycerin, which did not show any bacterial growth inhibition. On average, the drugs that showed the greatest antibacterial effects were 2% chlorhexidine and calcium hydroxide paste in camphorated paramonochlorophenol (CPMC) /glycerin and 0.12% chlorhexidine. It was concluded that 0.12% CHX as intracanal medication based on 2% CHX gel showed better antimicrobial action of the substances tested on bacterial species of endodontic infection.

Keywords: Intracanal medication; Auxiliary chemical substance; Antibacterial activity; Endodontic treatment.

Resumo

A eliminação da infecção intrarradicular é essencial para o sucesso do tratamento endodôntico. Este estudo avaliou os efeitos antibacterianos de nove diferentes formulações de medicações intracanaís e solução irrigadora, a base de hidróxido de cálcio (Ca(OH)₂), sulfato de cálcio (CaSO₄) e clorexidina (CHX) contra nove cepas bacterianas. Bactérias aeróbicas ou anaeróbicas facultativas foram cultivadas em caldo tripticase-soja e a cepa anaeróbia foi cultivada em infusão de cérebro-coração anaeróbia pré-reduzida esterilizada. A avaliação foi realizada por meio do teste de difusão em ágar em condições aeróbicas e anaeróbicas utilizando placas contendo ágar mitis-salivarius ou

ágar tripticase-soja. Os resultados mostraram que praticamente todas as substâncias testadas apresentaram alguma atividade antibacteriana, exceto o hidróxido de cálcio e o sulfato de cálcio quando associados apenas à glicerina, que não apresentaram inibição do crescimento bacteriano. Concluiu-se que a solução de clorexidina a 0,12% como medicação intracanal à base de gel de clorexidina a 2% apresentaram melhor ação antimicrobiana das substâncias testadas sobre as espécies bacterianas de infecção endodôntica.

Palavras-chave: Medicação intracanal; Substância química auxiliar; Atividade antibacteriana; Tratamento endodôntico.

Resumen

La eliminación de la infección intrarradicular es fundamental para el éxito del tratamiento endodóntico. Este estudio evaluó los efectos antibacterianos de nueve formulaciones diferentes de medicación intracanal y solución de irrigación, a base de hidróxido de calcio (Ca(OH)_2), sulfato de calcio (CaSO_4) y clorhexidina (CHX) contra nueve cepas bacterianas. Se cultivaron bacterias aeróbicas o anaeróbicas facultativas en caldo de tripticasa-soja y la cepa anaeróbica se cultivó en infusión de cerebro y corazón esterilizada anaeróticamente y reducida previamente. La evaluación se realizó mediante la prueba de difusión en agar en condiciones aeróbicas y anaeróbicas utilizando placas que contenían agar mitis-salivarius o agar tripticasa-soja. Los resultados mostraron que prácticamente todas las sustancias ensayadas tenían alguna actividad antibacteriana, excepto el hidróxido de calcio y el sulfato de calcio cuando se asociaron solo con glicerina, que no mostraron inhibición del crecimiento bacteriano. Se concluyó que la solución de clorhexidina al 0,12% como la medicación intracanal a base de gel de clorhexidina al 2% mostraron mejor acción antimicrobiana de las sustancias ensayadas sobre especies bacterianas de infección endodóntica.

Palabras clave: Medicación intracanal; Sustancia química auxiliar; Actividad antibacteriana; Tratamiento de endodoncia.

1. Introduction

The role of microorganisms in the etiology of pulpal and periradicular diseases was firstly observed approximately one century ago, and it has been demonstrated that the endodontic infections are mixed and polymicrobial, with a higher prevalence of anaerobic bacteria (Kakehashi, et al., 1965; Siqueira, et al., 2009). Because of the recognized role of bacteria in the induction and perpetuation of periradicular diseases, the eradication of root canal infection is paramount in endodontic therapy. Studies have revealed that the success rate of endodontic treatment is higher if the root canal is free from bacteria than if it is infected at the time of the root canal filling (Arruda, et al., 2018; Neves, et al., 2020; Zandi, et al., 2019).

Studies have demonstrated that endodontic infection can be reliably eradicated by using aseptic procedures, antimicrobial irrigating solutions, and an antimicrobial intracanal medicament between visits (Karatat, et al., 2020; Silveira, et al., 2007). The chemomechanical preparation promotes the elimination of most of the bacterial cells within the root canal system through both mechanical and chemical effects. Cleaning and disinfecting intracanal procedures are highly dependent on the mechanical and chemical effects of the preparation. Mechanical effects are generated by the instrument cutting motions and the flow and backflow of irrigant solution in the root canal. Chemical effects are provided by the irrigating solutions, which may have antimicrobial activity and/or tissue dissolving ability (Siqueira, 2001).

Although chemomechanical preparation eliminates most of the bacterial cells in the root canal, it has been shown that, in most cases, a small part of microbiota survives (Rodrigues, et al., 2017; Siqueira, et al., 2007a; Siqueira, et al., 2007b). Bacteria can be in areas distant from the main root canal, including isthmuses, ramifications, fins, and dentinal tubules. In these areas, they are unaffected by the instruments and irrigant solutions. Intracanal disinfecting medicaments can reach and eliminate residual microorganisms by remaining in the root canal system for more extended periods than irrigants (Alrahman, et al., 2020; Arruda, et al., 2018; Ricucci, et al., 2011).

Since the primary goal of the endodontic treatment of teeth associated with periradicular lesions is to eliminate the root canal infection, the purpose of this *in vitro* study was to compare the antimicrobial activity of potential and established intracanal medicaments and irrigating solutions.

2. Methodology

Medicaments and products tested

The medicaments and auxiliary chemical substances tested were as follows:

- a) Calcium hydroxide (Ca(OH)₂) /glycerin paste;
- b) Calcium hydroxide (Ca(OH)₂) /camphorated paramonochlorophenol(CPMC)/glycerin paste, hereafter referred to as CHPG. The CPMC/glycerin ratio of 1:1 (v:v);
- c) Calcium hydroxide (Ca(OH)₂) /camphorated paramonochlorophenol (CPMC) / glycerin paste, hereafter referred to as CHPG. The ratio of CPMC and glycerin 1:2 (v:v);
- d) Calcium hydroxide (Ca(OH)₂) / 0.12% chlorhexidine paste(CHX);
- e) 0.12% chlorhexidine (CHX) /zinc oxide (ZnO) paste;
- f) Calcium sulfate (CaSO₄) / 0.12% chlorhexidine (CHX) / glycerin paste. The CHX/glycerin ratio was 1:1 (v:v);
- g) Calcium sulfate (CaSO₄) / glycerin paste;
- h) 2% chlorhexidine (CHX) in natrosol gel containing a detergent (1.25% sodium lauryl-diethylene glycol ether sulfate);
- i) 0.12% chlorhexidine (CHX) solution.

Antibacterial Activity Assay

The bacterial strains utilized to test the antimicrobial properties of medicaments and irrigants were the following: *Fusobacterium nucleatum* subspecies *polymorphum* (ATCC 10953); *Streptococcus sobrinus* (ATCC 33478); *Streptococcus pyogenes* (ATCC 12344); *Streptococcus oralis* (ATCC 35037); *Streptococcus mitis* (own isolate); *Streptococcus intermedius* (own isolate); *Streptococcus constellatus* (own isolate); *Lactobacillus casei* (own isolate); and *Pseudomonas aeruginosa* (ATCC 10145).

Overnight cultures of the bacterial strains were used. Aerobic or facultative anaerobic bacteria were grown in trypticase-soy broth (TSB) (Difco, Detroit, MI, USA), and the anaerobic strain was grown in pre-reduced anaerobically sterilized brain heart infusion (BHI-PRAS) (Difco) broth supplemented with hemin (5 mg/ml) and vitamin K1 (1 mg/ml). Turbidity of the inocula, prepared in BHI-PRAS for anaerobes or in TSB for the other microorganisms, was adjusted to the turbidity of a 0.5 McFarland BaSO₄ standard (approximately 1.5 x 10⁸ colony forming units/ml).

Petri dishes containing trypticase-soy agar (TSA) (Difco) enriched with 5% defibrinated sheep blood and supplemented with hemin, and vitamin K1 were seeded with the anaerobic bacteria or the mixed culture (saliva). Plates containing Mitis-Salivarius agar were seeded with the test strains of *Streptococcus*. The other bacterial strains were inoculated onto the surface of TSA plates. Seeding was done using sterile cotton-tipped applicators that were brushed across the agar surfaces. Six wells of 5 mm depth and 6 mm diameter were punched in each agar plate and filled with freshly prepared medications. Irrigants were tested on separate plates using sterile paper dishes (6 mm in diameter) soaked in solutions. Plates were then left at room temperature by approximately 10 minutes to allow for the absorption of the inoculum. All the procedures were done in duplicate.

Agar plates inoculated with the mixed culture, or the anaerobic bacteria were incubated into anaerobic jars at 37°C for 5 days. Anaerobic conditions were obtained using the GasPak Plus generators (BBL, Becton-Dickinson Microbiology Systems, Cockeysville, MD, USA). Agar plates inoculated with the other microorganisms were incubated aerobically at 37°C for 24 to 48 hours. The antimicrobial effects of each material were determined by measuring the diameter of zones of inhibition in millimeters, obtained manually. The diameter of 6 mm served as the cut-off value for the pastes.

Data were analyzed using the Kruskal-Wallis test with tied ranks to allow a general view of the antimicrobial effectiveness of the test materials. The significance level was established at 5% (p<0.05).

3. Results and Discussion

Regarding the substances used as intracanal medication, the most pronounced zones of bacterial inhibition were observed for 2% CHX gel containing a detergent, followed by CHPG and calcium sulfate with CHX/glycerin. Calcium hydroxide or calcium sulfate with glycerin were the only medications that showed no antimicrobial effects on all tested species. The diameter of the bacterial inhibition zone for each tested substance against each bacterial species is shown in Table 1.

The antimicrobial activity of some drugs and chemical substances used in endodontics was evaluated according to the bacterial growth inhibition halo formed around them. The test used was the agar diffusion test, which allows for excellent contact between the analyzed substance and the region where bacteria may or may not proliferate, depending on the antimicrobial effect of each drug. The culture method allows to determine the susceptibility of microorganisms to antimicrobials and thus verify the effectiveness (Siqueira, et al., 2005a). All substances in this study were tested on species commonly found in primary intraradicular infection and secondary and/or persistent intraradicular infection (Roças, et al., 2011; Santos, et al., 2011; Siqueira, et al., 2005b).

Calcium hydroxide is the most common intracanal medication used in conventional endodontic treatment, and among the antimicrobial properties consist of alkaline pH having its action by direct contact with the bacteria (Rojas, et al., 2021). Intracanal medication between endodontic treatment sessions for teeth with apical periodontitis has shown high success rates regardless of the type of instrument used in the chemical-mechanical preparation (Neves, et al., 2020). Different calcium hydroxide pastes were proposed in this work. Pastes containing CPMC and CHX had the highest antimicrobial activity.

The antimicrobial effectiveness of CPMC against microorganisms involved in endodontic infection has been demonstrated (Ayhan, et al., 1999; Orstavik, et al., 1990; Siqueira, et al., 2008). This substance has bactericidal activity, damages the bacterial cytoplasmic membrane, denatures proteins, releases chlorine, and inactivates enzymes (Siqueira, et al., 1997). When associated with calcium hydroxide paste, CPMC works as an oily vehicle, being slowly released by the paste, reducing its toxicity. The bactericidal property of the paste is directly related to the CPMC concentration, as observed in the present study.

Calcium hydroxide associated only with glycerin was not able to inhibit bacterial growth in the agar diffusion test. The diffusion of calcium hydroxide is essential to exert its properties, which is observed in the blood-agar medium by forming a diffusion halo around the medicinal paste. However, the alkaline effects of calcium hydroxide can be buffered by components of the culture medium, inhibiting its action. Thus, this test has limitations in evaluating the effects of calcium hydroxide in an inert vehicle.

The antimicrobial activity of CHX is related to the fact that this cationic bisbiguanidine is a strong base, practically insoluble in water, and has permeability capacity in the bacterial cytoplasmic membrane, being slowly dispensed, culminating in a prolonged antimicrobial effect (Yan, et al., 2021; Alrahman, et al., 2020; Kalaiselvam, et al., 2019). When we combined 2% CHX with a surfactant, the antibacterial effect was excellent. Calcium sulfate and glycerin in a paste containing 0.12% CHX revealed a reduction of its antibacterial capacity, but it was higher than most pastes tested in this study. When we used 0.12% CHX associated with calcium hydroxide paste, the antimicrobial effect of the paste was lower than that of all other materials analyzed, possibly because of the test on calcium hydroxide. However, perhaps by raising the pH, the efficacy of CHX was reduced.

It should be emphasized that calcium sulfate associated only with glycerin did not exert any antimicrobial activity. However, replacing calcium hydroxide with zinc oxide when preparing the paste containing 0.12% CHX, the halo of inhibition increased significantly.

4. Conclusion

It was concluded that 0.12% chlorhexidine solution as an irrigant and Intracanal medication based on 2% chlorhexidine gel showed better antimicrobial action of the substances tested on bacterial species of endodontic infection. These substances demonstrate great potential for the antimicrobial activity for clinical use in cases of endodontic infection.

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