Antimicrobial and Chemical Study of MTA, Portland Cement, Calcium Hydroxide Paste, Sealapex and Dycal

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Introduction | Material and Methods | Results | Duscussion | Conclusions | Resumo | References

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The objective of this study was to investigate the antimicrobial action of mineral trioxide aggregate (MTA), Portland cement, calcium hydroxide paste (CHP), Sealapex and Dycal. The chemical elements of MTA and two Portland cements were also analyzed. Four standard bacterial strains: Staphylococcus aureus (ATCC 6538), Enterococcus faecalis (ATCC 29212), Pseudomonas aeruginosa (ATCC 27853), Bacillus subtilis (ATCC 6633), one wild fungus, Candida albicans (ICB/USP-562), and one mixture of these were used. Thirty Petri plates with 20 ml of BHI agar were inoculated with 0.1 ml of the experimental suspensions. Three cavities, each one measuring 4 mm in depth and 4 mm in diameter, were made in each agar plate using a copper coil and then completely filled with the product to be tested. The plates were pre-incubated for 1 h at environmental temperature followed by incubation at 37°C for 48 h. The diameters of the zones of microbial inhibition were then measured. Samples from diffusion and inhibition halos were extracted from each plate and immersed in 7 ml BHI broth and incubated at 37°C for 48 h. Analyses of chemical elements present in MTA and in two samples of Portland cement were performed with a fluorescence spectrometer Rx. The results showed that the antimicrobial activity of CHP was superior to those of MTA, Portland cement, Sealapex and Dycal, for all microorganisms tested, presenting inhibition zones of 6-9.5 mm and diffusion zones of 10-18 mm. MTA, Portland cement, and Sealapex presented only diffusion zones and among these, Sealapex produced the largest zone. Dycal did not show inhibition or diffusion zones. Portland cements contain the same chemical elements as MTA except that MTA also contains bismuth.

Key Words: calcium hydroxide, intracanal dressing, vehicles.

Introduction

Scientific investigations have been carried out to find products that have chemo-physical and biological properties that can induce tissue healing or replace dental structures that have been injured.

Calcium hydroxide (CH) has been selected as the optimal medication for several clinical situations due to its biocompatibility, stimulation for the formation of mineralized tissue and its antimicrobial action. The biologic and chemical effects of CH, its ionic dissociation into calcium and hydroxyl ions and its action on tissue and bacteria have determined important enzyme properties (Estrela et al., 1999, Holland et al., 1999). However, some of its properties, such as the physical and mechanical properties of solubility and resistance, have been the subjects of doubts.

Different products containing CH in their formulations have been commercialized and recommended for several clinical situations. Holland and Souza (1985) observed that Sealapex, which contains calcium oxide, encourages apical closure by means of cementum deposit. Recently, a new product MTA was suggested to seal off pathways of communication between the root canal system and the external surface of the tooth (Lee et al., 1993, Torabinejad et al., 1993, 1995a,b). Holland et al. (1999) studied the reaction of rat connective tissue to implanted dentin tubes filled with MTA or CH, and observed similar results. It is possible that the mechanism of action of MTA, encouraging hard tissue deposit, is similar to that of CH.

The purpose of this study was to investigate the antimicrobial action of MTA, Portland cement, CHP, Sealapex and Dycal, and analyze the chemical elements of the MTA and two samples of Portland cements.

Material and Methods

Four standard bacterial strains obtained from the American Type Culture Collection were used: *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633). One wild fungus, *Candida albicans* (ICB/USP-562), isolated by the Laboratory of Biological Science (University of São Paulo, SP, Brazil), was included in the study.

The products tested were: Mineral Trioxide Aggregate® (MTA, Dentsply, Tulsa Dental, Tulsa, OK, USA), Portland cement (Cia. Portland Cement Itaú, Itaú de Minas, MG, Brazil), calcium hydroxide paste (CHP, P.A., Quimis, Mallinkrodt Inc., St. Louis, MO, USA + saline solution), Sealapex® (Sybron-Kerr, Romulus, MI, USA), and Dycal® (Dentsply, Petrópolis, RJ, Brazil).

Agar diffusion test

The strains were inoculated in 5 ml of brain heart infusion (BHI, Difco Laboratories, Detroit, MI, USA) and incubated at 37°C for 24 h. In order to prepare the experimental suspensions, the biological indicators cited above were cultivated on the surface of BHI agar (Difco Laboratories), following the same incubation conditions; microbial cells were re-suspended in saline to obtain a final concentration of approximately 3 x 10^8 cells/ml, similar to that of tube #1 of the MacFarland scale. One ml of each of these pure suspensions was used to obtain a mixture of the test microorganisms.

The substances were studied using the agar diffusion test. Thirty Petri plates with 20 ml BHI agar were inoculated with 0.1 ml of the experimental suspensions, using sterile swabs that were brushed across the medium, obtaining growth in junction. Three cavities, measuring 4 mm in depth and 4 mm in diameter, were made in each agar plate using a copper coil and then completely filled with the product to be tested. The plates were pre-incubated for 1 h at environmental temperature followed by incubation at 37°C for 48 h. The diameters of the zones of microbial inhibition were measured. Samples from diffusion and inhibition halos were extracted from each plate and immersed in 7 ml BHI broth and incubated at 37°C for 48 h. Positive and negative controls were done, maintaining the plates with and without inoculum, for the same period and under identical incubation conditions. All assays were carried out under aseptic conditions. Tests were performed in triplicate.

Fluorescence spectrometry Rx

A second chemical test was performed in order to analyze chemical elements present in MTA and two samples of Portland cement, one Itaú brand (Cia. Portland Cement Itaú, Itaú de Minas, MG, Brazil), and the other Liz brand (Cia. Portland Cement Liz, Vespasiano, MG, Brazil). A fluorescence spectrometer Rx was used for the chemical analysis (Carl Zeiss, Mod. VRA-30, Germany). Tests were performed in triplicate.

Results

The results of the antimicrobial effect of experimental products are shown in Table 1. Samples that were extracted from plates with diffusion zone and then inoculated in BHI and incubated at 37°C for 48 h showed the presence of growth and the inhibition zone showed the absence of growth.

The antimicrobial action of CHP on all microorganisms tested was superior to that of MTA, Portland cement, Sealapex and Dycal, showing inhibition zones ranging from 6 to 9.5 mm and diffusion zones ranging from

10 to 18 mm. MTA, Portland cement, and Sealapex presented only diffusion zones and among these, Sealapex produced the largest zone (10 to 18 mm). Diffusion zones produced by Portland cement and MTA on *S. aureus, P. aeruginosa* and the mixture ranged from 7 to 8 mm, respectively; *B. subtilis* and *C. albicans* were similar for these products. Portland cement showed a diffusion zone of 16 mm and MTA a diffusion zone of 14 mm on *E. faecalis.* Dycal did not show inhibition or diffusion zones.

Chemical analyses of MTA and Portland cements are shown in Table 2. These analyses showed that Portland cements contain the same chemical elements (plus others) as MTA except that MTA also contains bismuth.

Discussion

Estrela et al. (2000) tested CH antimicrobial potential by the direct exposure test and agar diffusion test. They concluded that, collectively, both tested procedures may be useful to establish the CH antimicrobial spectrum, thus, improving infection control protocols.

The diffusion test in agar is largely used in microbiology. However, it does not establish reliable parameters to compare substances with different chemical characteristics such as dissociation and diffusion capabilities in semi-solid medium, not expressing their real antimicrobial effect (Estrela et al., 1999). Advantages and limits of the agar diffusion test have also been discussed in other studies (Tobias, 1988; Pumarola et al., 1992; Weiss et al., 1996; Fuss et al., 1997).

Torabinejad et al. (1995b) investigated the antibacterial effect of some root end filling materials by means of the agar diffusion test and observed that MTA had no antibacterial activity against *S. faecalis*, *S. aureus* and *B. subtilis*, and no effect on any of the strict anaerobic bacteria. The present study showed antimicrobial results similar to those of Torabinejad et al. (1995b). The results obtained with MTA on *S. aureus, E. faecalis* and *B. subtilis* were identical to those obtained using Portland cement. The diffusion zone of MTA and Portland cement did not show meaningful differences. Sealapex presented only a diffusion zone in agar (10 a 18 mm), not showing an inhibition zone.

Tagger et al. (1988) investigated the release of calcium and hydroxyl ions from set endodontic sealers containing CH, and reported that disintegration of the pellets of Sealapex indicated that solubility might be the price for increased activity. Sealapex favored the apical healing process (Holland and Souza, 1985; Holland et al., 1998).

Chemical evaluation of elements present in MTA and Portland cements by fluorescence spectrometer Rx is shown in Table 2. In this analysis, it was observed that Portland cements contain the same chemical elements as MTA except that MTA also contains bismuth. In chemical assays of a Portland cement, the components were present according to the following average percentages: CaO (58.5%), SiO_2 (17.7%), Al_2O_3 (4.5%), MgO (3.3%), SO_3 (3.0%), Fe_2O_3 (2.9%), K_2O (0.9%), Na_2O (0.2%).

Weidmann et al. (1994) studying the ingredients of Portland cement, reported that the term "cement" is generic and is applicable to all binders that are used in concrete. The constituents of Portland cement are minerals, which exist as multicomponent solid solutions. The solutes or impurities are important because they can affect the crystal structures and/or their ability to react. Despite this, it is usual to treat the main constituents as chemical compounds and to label them as if they were mixtures of oxides, but using a shorthand nomenclature. This involves replacing the usual chemical formula for an oxide with a single letter, such as CaO, SiO_2, Al_2O_3, Fe_2O_3, H_2O, SO_3. Portland cement has various constituents but the main ones are: tricalcium silicate ($3CaO.SiO_2$), dicalcium silicate ($2CaO.SiO_2$), tricalcium aluminate ($3CaO.Al_2O_3$), tetracalcium aluminoferrite ($4CaO.Al_2O_3.Fe_2O_3$), hydrated calcium sulphate ($CaO.SO_3.2H_2O$), alkali oxides and other constituents. The basic constituents of Portland cements are similar (Van Vlack, 1973; Callister, 1991; Weidmann et al., 1994). Small differences can be noticed depending on the manufacturer and the location of the source of mineral extraction.

During the first minute, the pH of Portland cement rises from that of the starting water, 7, to a pH of 12.3 and continues rising to a maximum pH of 12.9 after three hours. The potassium and sodium ions present come clearly from the minor alkali oxide constituents, which also provides an additional source of hydroxyl ions.

The main sources of hydroxyl ions are the O^{2-} ions produced by the linking of tetrahedra silicate and by the dissolution of CaO (Weidmann et al., 1994).

Torabinejad et al. (1995a) determined the chemical composition and pH of a new root-end filling material. The main components of MTA powder are tricalcium silicate, tricalcium aluminate, tricalcium oxide, and silicate oxide. In addition, there are small amounts of a few other mineral oxides that are responsible for the chemical and physical properties of this aggregate. Bismuth oxide powder is added to make the aggregate radiopaque. The pH of MTA after mixing was 10.2, and rose to 12.5 within 3 h; thereafter, it remained constant.

Holland et al. (1999) compared the reaction of rat connective tissue to implanted dentin tubes with CH and MTA. Granulations birefringent to polarized light could be observed with CH. These granulations are calcite crystals originating from the reaction of the calcium from the CH with the carbon dioxide from the pulp tissue. In this experiment, the authors observed the crystals and a calcified tissue that resembled a barrier at the opening of the tubes. Similar results were observed with MTA.

The fact that the main components found in MTA are also present in Portland cement can justify why the results of the antimicrobial activity tests were similar.

This investigation confirms the necessity of further comparative studies of MTA, Portland cement and calcium hydroxide because of the similarities between the mechanisms of action of calcium hydroxide and MTA, in agreement with the results obtained by Holland et al. (1999), and the presence of the same chemical elements in MTA and Portland cement.

Conclusions

1.Antimicrobial activity of calcium hydroxide over all microorganisms studied was superior to that of MTA, Portland cement, Sealapex and Dycal, showing inhibition zones that ranged from 6 to 9.5 mm and diffusion zones ranging from 10 to 18 mm. MTA, Portland cement, and Sealapex showed only diffusion zones and Sealapex produced the largest zone. Dycal did not show inhibition or diffusion zones.

2.Portland cements contain the same chemical elements as MTA except that MTA also contains bismuth.

Resumo

Estrela C, Bammann LL, Estrela CRA, Silva RS, Pécora JD: Estudo antimicrobiano e químico do MTA, cimento Portland, pasta de hidróxido de cálcio, Sealapex e Dycal. Braz Dent J 11(1): 3-9, 2000.

O objetivo deste estudo foi investigar a ação antimicrobiana do agregado trióxido mineral (MTA), cimento Portland, pasta de hidróxido de cálcio (CHP), Sealapex e Dycal. Os elementos químicos do MTA e de dois cimentos Portland também foram analisados. Quatro cepas bacterianas: *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), um fungo, *Candida albicans* (ICB/USP-562), e uma mistura destes foram usados. Trinta placas de Petri com 20 ml de BHI agar foram inoculadas com 0,1 ml da suspensão experimental. Três cavidades, cada uma medindo 4 mm de profundidade e 4 mm de diâmetro, foram feitas em cada placa usando um cilindro de cobre e, em seguida, completamente preenchidas com os produtos a serem testados. As placas foram pré-incubadas por 1 h em temperatura ambiente e, a seguir, incubadas a 37°C por 48 h. Os diâmetros das zonas de inibição microbiana e de difusão foram medidos. Amostras dos halos de difusão e inibição foram extraídos de cada placa e imersos em 7 ml de caldo BHI e incubados a 37°C por 48 h. A análise química dos elementos presentes no MTA e em duas amostras de cimentos Portland foi feita com um Espectrômetro de Fluorecência de Raios-X. Os resultados mostraram que a atividade antimicrobiana da pasta de hidróxido de cálcio foi superior a todas as outras substâncias (MTA, cimento Portland, Sealapex e Dycal), sobre todos os microrganismos testados, apresentando zonas de inibição com 6-9,5 mm e zonas de difusão com 10-18 mm. O MTA, o cimento Portland e o Sealapex apresentaram somente zonas de difusão e, dentre estes, o Sealapex apresentou a maior zona. O Dycal não apresentou halos de inibição, nem de difusão. Os cimentos Portland contém os mesmos elementos químicos que o MTA, com a exceção que o MTA também apresenta, na sua constituição, o bismuto.

Unitermos: hidróxido de cálcio, medicação intracanal, veículos.

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BACK TO CONTENTS