Evaluation of Antimicrobial Effect of Mineral Trioxide Aggregate, Calcium Hydroxide and Calcium Hydroxide with Iodoform on Microbial Growth in Vitro

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Background: The success of treatment of a young permanent tooth with pulp necrosis appropriate each stage of development depends primarily on the complete sterilization of infected root canals using specific endodontics drugs capable of destroying pathogenic microorganisms, especially obligate and facultative anaerobic bacteria.
Aim: The purpose of this research is to evaluate the antimicrobial effect of the three most used intraradicular substances, respectively mineral trioxide aggregate, calcium hydroxide and calcium hydroxide with iodoform on anaerobic bacteria isolated from infected root canals and also on pure bacterial species, namely Staphylococcus Aureus , Enterococcus Faecalis and Candida Albicans, cultures coming from the collection of the microbiology laboratory.
Material and methods: Anaerobic species grown on specific culture medium were prepared in the form of bacterial suspension using standardized system of 0, 5 McFarland turbidity diluted by half. The three drugs tested were used in different concentration and to each of them was added 1 ml of bacterial suspension obtained previously. From each sample obtained were made insemination on specific anaerobic medium and interpretation of results was made by comparing the number of bacterial colonies grown.
Results: Following this study MTA has failed to demonstrate superiority over calcium hydroxide in terms of antibacterial properties results showing that each substance tested has elective action on bacterial species.
Conclusion: All endodontic materials tested have shown antimicrobial properties able to effectively sterilize infected root canals but they compared different actions on bacterial species taken to study depending on the amount of active substance tested.

Keywords: endodontic materials, antimicrobian effect, anaerobic bacteria

Introduction

In the apical and periapical zone of immature teeth are unlimited repair potentials, able to induce hard tissue barrier formation (apexification) even with total loss of pulp vitality. The success of this method requires standard endodontics procedures to neutralize bacterial toxins followed by the application of drugs intraradicular till the apical area. It is possible that these substances are not essentially stimulus for apexification but the complete sterilization of root canals creates appropriate conditions to induce barrier formation.

The main cause of pulp necrosis is represented by anaerobic bacteria and their toxins, immunologic agents, tissue debris and products of tissue necrosis [1]. Calcium hydroxide (CH) is at this moment the intraradicular medication of choice in treatment of pulp necrosis of immature teeth because of its ability to induce apexification and also its known antimicrobial effects.

Antimicrobial activity is due to hydroxyl ions which are released gradually giving a high alkalinity preparations [2]. It can be mixed with other substances (saline solution, antibiotics, barium sulfate) but especially with iodoform (I), a powder with bright hexagonal crystals of lemon yellow colour, little soluble in water, soluble in alcohol and ether. It decomposes releasing iodine in nascent state with a high reactivity by precipitating proteins and oxidizing essential enzymes [3]. The major disadvantage of calcium hydroxide is higher resorbence which requires periodic replacement till the complete root formation.

An alternative to calcium hydroxide came with the discovery of a new endodontic material respectively mineral trioxide aggregate (MTA) which was developed by Dr. Torabinejad at Loma University in 1990. Since then it has been widely tested and the results confirm that MTA is a material with excellent biological and physical properties such as antimicrobial effect, citotoxicity, cement deposition and hard tissue barrier formation [4].

The purpose of this study is to compare the antimicrobial effect of MTA, calcium hydroxide and calcium hydroxide mixed with iodoform, substances used for the treatment of pulp necrosis in immature teeth on anaerobic bacteria, some isolated from infected canals and on facultative anaerobic strains obtained from microbiology laboratory stock.

Materials and methods

The study was performed on obligate anaerobic bacteria isolated from infected canals by us and also on facultative anaerobic strains obtained from microbiology stock: Staphylococcus aureus (25293), Enterococcus faecalis (CBS 562), Escherichia coli and the only fungus Candida albicans. The prelevation and isolation of anaerobic bacte-

ria from infected root canals was done with sterile paper points immersed in canal secretions and kept to grow three days in a specific liquid anaerobic culture medium (thioglycollic) at 37 °C in anaerobiosis Generbag system.

After that another bacterial inoculation was done on a solid culture Schaedler medium for anaerobic supplemented with blood. The medium plates were kept 24 hours at 37°C in Generbag system followed by bacterial suspensions preparation. All bacterial suspensions used for testing the three endodontic substances were obtained using a 0, 5 concentration Mc Farland turbidity standard method diluted to half. The McFarland 0.5 standard, base on optical density, has particular application in the preparation of bacterial cultures for performing antimicrobial susceptibility testing and the advantage is that none incubation time or equipment is needed to estimate bacterial numbers.

Bacterial suspension prepared was obtained using a thioglycollic medium for obligate anaerobic species and a Broth medium for facultative anaerobic bacteria. The three substances tested were: mineral trioxide aggregate, calcium hydroxide and the combination of calcium hydroxide with iodoform, all used in state of powder diluted in bacterial suspension.

To determine the minimum inhibitory concentration (MIC) we have used different quantity starting with 2 mg, 4 mg, 6mg, 8 mg, 10 mg, 12 mg, 14 mg, 16 mg and 18 mg of each tested substances using an electronic balance. The combination calcium hydroxide-iodoform was 1:1 mixed to obtain the same quantity with the other substances. The weighted quantities of the three substances were inoculated with 1 ml of each bacterial suspension in test tubes for each bacterial strains. One test-tube remained for control containing just 1 ml of each bacterial suspension. The test-tubes were kept for 48 hours at 37°C and obligatory anaerobic bacteria were cultured in anaerobic condition using Generebag system. From each test-tubes sample the bacterial strains were inoculated on agar medium and after 24 hours incubation appeared the first results of this study.

The interpretation of the results was done comparing the number of bacterial colonies developed on each sample plate inoculated with different quantities of endodontic substances with number of colonies from control group.

Results

The results obtained in this study showed antibacterial effectiveness of the three endodontic substances tested on bacterial species examined demonstrating the different actions depending on the amount used. The antimicrobial effects on bacterial species studied and the minimum inhibitory concentration of each substance tested are listed in the tables I–IV.

Discussions

The final results obtained from this study confirm the excellent antibacterial properties of CH and MTA, the materials of choice at this moment in the treatment of pulp necrosis of immature teeth. Gergely et al. 5 reported that calcium hydroxide is recommended most frequently in the treatment of persistent exudation from infected canals at teeth with apical periodontitis. CH is still the most used antimicrobial endodontic material because of its high pH value (~ 12). The basic principle action of calcium hydro-

Table I. Antimicrobian effect of CH, CH+I and MTA on Staphylococcus aureus

S. aureus	Control	2 mg	4 mg	6 mg	8 mg	10 mg	12 mg	14 mg	16mg	18 mg
CH	++++++	++++++	++++	++	0	0	0	0	0	0
CH+I	++++++	++++++	++++++	++++	+++	+++	0	0	0	0
MTA	++++++	+++++	+++	0	0	0	0	0	0	0

Table II. Antimicrobian effect of CH, CH+I and MTA on Obligate Anaerobe Bacteria

Anaerobes Bacteria	Control	2 mg	4 mg	6 mg	8 mg	10 mg	12 mg	14 mg	16 mg	18 mg
CH	++++++	+++++	+++++	++++	++++	++++	++++	++++	+++	0
CH+I	++++++	+++++	+++++	++++	++++	++++	+++	+++	++	++
MTA	++++++	++++++	+++++	++++	++++	++++	++++	++++	++++	++++

Table III. Antimicrobian effect of CH, CHI and MTA on Enterococcus Faecalis

E. faecalis	Control	2 mg	4 mg	6 mg	8 mg	10 mg	12 mg	14 mg	16mg	18 mg
CH	++++++	+++++	+++++	++++	++++	++++	++++	+++	0	0
CH+I	++++++	++++	++	0	0	0	0	0	0	0
MTA	++++++	+++++	+++++	++++	++++	+++	++	0	0	0

Table IV. Antimicrobian effect of CH, CHI and MTA on Candida albicans

C. albicans	Control	2 mg	4 mg	6 mg	8 mg	10 mg	12 mg	14 mg	16 mg	18 mg
CH	++++++	++++	+++	0	0	0	0	0	0	0
CH+I	++++++	+++++	+++++	++++	+++	0	0	0	0	0
MTA	++++++	+++++	++++	+++	0	0	0	0	0	0

xide is the ionic dissociation into hydroxyl ions and calcium ions with effect on microorganisms and tissue healing [3]. Estrela et al. 6 studying the mechanism of action of hydroxyl ions on microbial enzymes reported that changes in the transport of nutrients and in the structure of organic components are responsible for its destruction. They suggested the hypothesis of an irreversible bacterial enzymatic inactivation under extreme conditions of pH for a long period of time and also a temporary bacterial enzymatic inactivation with the restoration of normal activity when pH returns to the ideal level for enzymatic activity.

In our study MIC for Staphylococcus Aureus of the MTA is less than that of CH or combined with iodoform and comparing calcium with calcium/iodoform mixture, iodoform does not seem to enhance the antibacterial action of calcium on Staphylococcus Aureus (Table I). Several works have studied the combination of calcium hydroxide with other substances to improve some properties. Kaiser was the first that mixed calcium hydroxide with another substance to induce an apical barrier formation. This method was described step by step in 1966 by Frank and also the four types of apical barrier obtained. All over the years pastes containing iodoform were exhaustively indicated as antiseptics due to iodine release in nascent state in contact with secretions of infected canals. The researches suggested that a 40 % iodoform added to calcium hydroxide increase the radio opacity and the antimicrobial effect, the pastes with iodoform maintain a soft consistency of material so that soluble calcium hydroxide remain active for a long time in root canals [7].

Results obtained from this study showed an increased antimicrobial effect of the combination calcium/iodoform only on Enterococcus faecalis compared with other substances (Table III). It is possible that low action of iodoform in vitro to be inhibited by its small solubility. Aydos and Milano [8] questioned the antiseptic ability of iodoform for use in root canal. They concluded that iodoform provides radio opacity of calcium hydroxide pastes and has no antibacterial action in vitro.

Evaluating the antibacterial activity of calcium hydroxide containing different amounts of iodoform on obligate anaerobic bacteria, Siqueira et al. [9] concluded that the addition of iodoform did not influence the bacterial properties. Also, Estrela et al. [3], using two experimental methods (agar diffusion test and direct exposure test) showed that iodoform paste presents antimicrobial ineffectiveness for the agar diffusion test on all biological microorganisms and for direct exposure test on B. subtilis and on mixture.

Another recent study showed that while calcium hydroxide was unable to kill Enterococcus faecalis in the dentine, calcium hydroxide combined with iodine potassium iodide (IKI) and chlorhexidine (CHX) effectively disinfected the dentine [10]. Antimicrobial action of calcium hydroxide on obligate anaerobic bacteria isolated from infected canals proved more effective than MTA's, the MIC was 14 mg CH compared to 18 mg of MTA's MIC (Table II). Isolated anaerobic bacterial species identification required by us on the macroscopic appearance of cultures but also on the microscopic appearance of the smear obtained from bacterial culture showed predominance of Gram + bacillus species growing in the colony type S (smooth) round, convex and shiny without hemolysis [11].

The calcium hydroxide-based filling material Apexit, which is often used in endodontic practice, was evaluated for its antibacterial and antifungal effects against microorganisms isolated from oral cavity. The study showed that Apexit inhibited Gram negative bacteria more effectively than Gram positive ones but had none or a very weak inhibitory effect on Candida Albicans [12].

Furthermore, following this research, MTA has demonstrated its antimicrobial qualities bacterial species under study but has not shown superiority over calcium hydroxide. The lower effect of MTA on anaerobic obligatory bacteria isolated from infected canals may be due also to reactions between components of thioglycollic medium that circulated these bacteria and mineral components of MTA especially since after incubation, all samples containing MTA presented a brown envelope.

MTA is a powder of fine hydrophilic particles consisting of compounds of tricalcium silicate, tricalcium oxide, tricalcium aluminates and silicate oxide [13]. Several studies have reported that MTA is similar to commercial Portland cement which represent 80 % and the rest of 20 % is bismuth oxide added for radio-opacity [14]. Both materials (PC and MTA) are formed by hydration a calcium silicate gel and calcium hydroxide in ratio 4:1, which can explain the similar mechanism of MTA and calcium hydroxide. Concentration of hydroxyl ions (pH) is 10 after mixing with distilled water increased after 3 hours at 12 which guarantees the long lasting microbial effect and unfavorable environment for microorganisms growth.

The antibacterial effects of gray-colored MTA (GMTA) and white-colored MTA (WMTA) against Enterococcus faecalis and Streptococcus sanguis were assessed in vitro using the tube dilution test. It appears that the susceptibility of E. faecalis and S. sanguis to MTA were different and that GMTA requires lower concentrations than WMTA to exercise the same antibacterial effect against each of the microorganisms tested [15].

Effect on Candida Albicans of MTA as shown in the study is lower than that of calcium hydroxide, MIC is 8 mg for MTA and 6 mg for CH and the addition of iodoform to CH failed to potentate its antimicrobial effect, MIC of CHI is 10 mg. (Table IV). Al-Nazhan et al. 16 tested in a study in vitro the antifungal effect of mineral trioxide aggregate (MTA) using a tube-dilution test. Results showed that the freshly mixed MTA was effective in killing the tested fungi after 1 day of contact, whereas the 24-h set MTA was effective after 3 days of incubation. It was concluded that MTA (freshly mixed and 24-h set) was effective against C. albicans. Regarding association of MTA with calcium hydroxide in treatment of immature

permanent teeth several studies showed that the regeneration of periapical tissues appears in a shorter period and the healing is complete [17]. A comparative in vitro study demonstrated that MTA and Portland cement have similar action on Pseudomonas aeruginosa. More, it showed that calcium hydroxide paste has a superior antimicrobial effect comparing with MTA and PC [18].

The results of another study showed that the 7-day dressing with calcium hydroxide efficiently eliminated bacteria which survived biomechanical instrumentation of the canal, while the 10-minute application was ineffective [19]. Therefore, the treatment protocol of gangrene in immature teeth using technique "one step apexification" is indicate to apply for 7 days in radicular canals a preparation based on CH.

Conclusions

The results of this study demonstrate the antibacterial qualities of all substances tested, each with a minimum inhibitory concentration on different bacterial species depending on which test was performed. In practice the concentration of active substance in preparation used is much higher than that in the study, so we can say that the antibacterial effect is guaranteed.

Following this study, MTA has failed to demonstrate superiority over calcium hydroxide in terms of antibacterial properties, results showing that each substance tested has elective action on bacterial species. The association between calcium hydroxide and iodoform did not increase the antibacterial effect, except the Enterococcus faecalis over which the combination was more efficient than MTA or CH. In practice, all this substances can be used alternately and combined in endodontic therapy especially for young permanent teeth may, increasing the chances of healing in complicated cases and thus provide conditions for apexification.

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