An *in vitro* Study of the Antimicrobial Activity of Mineral Trioxide Aggregate and of Calcium Hydroxide on Certain Species of Obligate or Facultative Anaerobic Bacteria

A comparative evaluation on young permanent teeth

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The success of treatment in case of lost pulp vitality depends on the antimicrobial ability of specific endodontic materials. We compared the antimicrobial effect ofmineral trioxide aggregate and calcium hydroxide on anaerobic bacteria isolated from infected root canals, as well as on two certain pure species of facultative anaerobic bacteria. After the incubation period, in a liquid culture medium under strictly anaerobic conditions the bacterial suspensions were prepared. Both materials were used in the form of diluted powder in the bacterial suspension. The antimicrobial effect was evaluated on the basis of the minimum inhibitory concentration, defined as the lowest concentration of an active substance that inhibits bacterial growth. The results showed that both materials have almost similar antimicrobial activity due to their chemical composition but calcium hydroxide has retained supremacy with main indication in apexification process.

Keywords: calcium hydroxide, mineral trioxide aggregate, anaerobic bacteria, young permanent teeth

Young permanent teeth have unlimited repair and induction mechanisms, which enable apical sealing after complete loss of pulp vitality. This depends largely on the creation of conditions favourable to the induction of the apical barrier, and on the debridement and removal of potentially irritating bacterial toxins, respectively. Anaerobic bacteria and the toxins released by them, immunological agents, debris and products of tissue necrosis represent the main cause of necrosis [1].

The material of choice used successfully in these cases is calcium hydroxide which, through its antibacterial and apexification induction properties, is capable of ensuring an increased opportunity for the functional recovery of the affected tooth. Its antimicrobial effects are due to the strongly alkaline properties given by the hydroxyl ions, which are released gradually. It can be used in combination with many substances, such as camphorated p-monochlorophenol, sterile saline, distilled water, anaesthetic solutions, chlorhexidine, antibiotics, barium sulphate, but especially with iodoform, a powerful antiseptic [2]. The major disadvantage of calcium hydroxide is its dissolving nature, which requires periodic replacement in the canals until the completion of the process of apexification.

Mineral trioxide aggregate (MTA) is an alternative to the calcium hydroxide therapy, thanks to its excellent biological and physical qualities, evidenced by its antimicrobial effects, reduced cytotoxicity, stimulation of the cement depositing and periapical hard tissue formation [3]. MTA is a powder consisting of a hydrophilic mixture of mineral oxides, such as: SiO_2 (*silicon dioxide*), K_2O (*potassium oxide*), Al_2O_3 (*aluminium oxide*), Na_2O (*sodium oxide*), Fe_2O_3 (*ferric oxide*), CaO (*calcium oxide*), MgO (*magnesium oxide*) [4]. Several studies have shown that MTA is similar to the Portland cement (80% of the composition), but, in addition, it contains 20% of bismuth oxide, with radiopacity effect [5]. Through hydration, both of them produce hydrated calcium silicate gel and calcium hydroxide in a ratio of 4:1. This could explain the similar mechanism of action of MTA and calcium hydroxide.

The aim of this study is to comparatively evaluate the antimicrobial effects of MTA and calcium hydroxide on anaerobic bacteria isolated from infected root canals, as well as on certain pure species of facultative anaerobic bacteria.

Experimental part

Material and method

For the microbiological sampling, the informed consent of the subjects included in the study and of their dependents was obtained.

Isolation of the anaerobic bacteria. Preparation of the bacterial suspensions

The tests were carried out on *obligate anaerobic* microorganisms, isolated from the root canals of some young permanent teeth with gangrene, as well as on pure cultures of facultative anaerobic bacteria, on *Staphylococcus aureus* (*25293*) and *Enterococcus faecalis*

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(292,212), respectively, which came from the culture collection of the Microbiology Laboratory of UMF Targu-Mures.

The sampling derived from 10 subjects aged 7-12 years who presented clinical and radiological symptoms of complicated gangrene on a YPT. For the sampling, sterile paper points were used, which were immersed for a few minutes in the secretions from infected root canals, before the beginning of the endodontic treatment.

Subsequently, they were placed in a liquid culture medium specific to anaerobes (thioglycollate broth) and were kept for 3 days at 37° C under strictly anaerobic conditions, using *Generbag-Mediclim* system. The seeding was done on culture medium specific to anaerobes, and Shaedler enriched blood agar, respectively, and were left for 24 h under conditions of anaerobiosis.

Following the incubation period, the bacterial suspensions were prepared, using the McFarland standard turbidity system of 0.5 diluted to half, on which the two endodontic materials were tested. For the preparation of bacterial suspensions in the case of anaerobic bacteria, the liquid thioglycollate was used, as culture medium, and in the case of facultative anaerobic bacteria – tomato juice. Both were used in the form of diluted powder in the bacterial suspension. The antimicrobial effect was evaluated on the basis of the minimum inhibitory concentration, defined as the lowest concentration of an active substance that inhibits bacterial growth. Thus, different amounts of the materials were used, namely 2, 4, 6, 8, 10, 12, 14, 16 and 18 mg and only 1mL of the bacterial suspension was added in a tube, thereby obtaining the control sample.

The samples thus prepared were left for 48 h at 37° C and, after the incubation of each sample, the seeding on blood agar culture medium was performed, the interpretation of results being carried out after the incubation period of 24 h.In order to facilitate the interpretation of the results, the seeding was performed on circular sectors.

Results and discussions

The interpretation of the results was carried out by comparing the number of bacterial colonies grown on each sector of the circle, following the seeding with bacterial suspensions at different concentrations (table 1).

The results comprised in table 1 show the antimicrobial qualities of calcium hydroxide and of MTA on specific microorganisms derived from pulp infections in permanent teeth, being considered, from this point of view, the materials of choice in the treatment of gangrene in children. The results show that the antimicrobial action of calcium hydroxide on anaerobic bacteria is more effective than that of MTA, the minimum inhibitory concentration being of 14 mg, compared to 18 mg of the MTA. In the present study, the reduced MTA effect on obligate anaerobic

Table 1

THE ANTIMICROBIAL EFFECT ON THE BACTERIAL SPECIES UNDER INVESTIGATION, AS WELL AS THE MINIMUM INHIBITORY CONCENTRATION OF EACH ACTIVE SUBSTANCE

Chemical composition of the material	**Mineral trioxide aggregate** (silicon dioxide, potassium oxide, aluminium oxide, sodium oxide, ferric oxide, calcium oxide, magnesium oxide)			**Calcium hydroxide** (slaked lime, obtained from the interaction between calcium oxide and water)		
Species of bacteria	**Obligate anaerobic bacteria**	**Staphylococcus Aureus**	**Enterococcus Faecalis**	**Obligate anaerobic bacteria**	**Staphylococcus Aureus**	**Enterococcus Faecalis**
Control	••••••	••••••	••••••	••••••	••••••	••••••
2 mg	••••••	•••••	•••••	•••••	••••••	•••••
4 mg	•••••	•••	•••••	•••••	••••	•••••
6 mg	••••	0	••••	••••	••	••••
8 mg	••••	0	••••	••••	0	••••
10 mg	••••	0	•••	••••	0	••••
12 mg	••••	0	••	••••	0	••••
14 mg	••••	0	0	••••	0	•••
16 mg	••••	0	0	•••	0	0
18 mg	••••	0	0	0	0	0

•1-10 col $10^3 – 10^4$ CFU/mL; ••10-20 col $10^4 – 2x10^4$ CFU/mL; •••20-50 col $2x10^4 – 5x10^4$ CFU/mL; ••••50-100 col $5x10^4 – 10^5$ CFU/mL; ••••• 100-200 (semiconfluent) > 10^5 CFU/mL; ••••••confluent

bacteria may be due to some inhibitory reactions resulting from some environmental components of thioglycolate, in which the bacterial species and the mineral components of the MTA were employed.

Gergely JM. et al. [6] have shown that calcium hydroxide is frequently recommended in the treatment of periapical abscess, which is due to its strongly alkaline *p*H, of about 12, the antibacterial effect being the result of its dissociation into hydroxyl ions and calcium ions, having, at the same time, a healing effect on damaged tissues as well. In the case of MTA, too, its antimicrobial activity has been associated with a high *p*H level. Torabinejad et al. have noted that the initial *p*H is 10.2, which rises to 12.5 after 3 h. It is known that *p*H values of 12 can inhibit the growth of most microorganisms, including resistant bacteria such as Enterococcus faecalis [7].

In this study we have noticed that the effect on E. Faecalis is stronger in the case of MTA with a minimum inhibitory concentration of 14 mg, compared with calcium hydroxide, which inhibits the growth at a value of 16 mg. Also, it can be observed that the antimicrobial activity of MTA on *Staphylococcus Aureus* is stronger than that of calcium hydroxide, the minimum inhibitory concentration being, in this case, 6 mg compared to 8 mg in the case of calcium hydroxide. The antimicrobial effect of MTA has been demonstrated by other researchers in different studies, while others have shown that MTA has no antibacterial effect on S. aureus, E. coli and C. albicans, although the same kind of MTA has been used. The different species of microorganisms tested and the different method of determining the antimicrobial activity might explain these different results [8-13].

Because the success rate in inducing the apexification phenomenon depends on complete root canal sterilization, these results could explain the cause of failure in some cases of gangrene in young permanent teeth. In an *in vivo* study, Beslot-Neveu et al. [14] have shown that, in the case of an immature tooth necrosis, MTA is not better than calcium hydroxide as far as the rate of the formation of calcified barrier is concerned, but MTA is capable of producing a biological barrier in less than 6 months. Annamalai S. and J. Mungara [15] have found a success rate of 100% both clinically and radiographically in the case of applying an apical MTA plug to an immature tooth, 86.6% of the cases resulting in apical closure and 30% in increased root.

Conclusions

The results of this study join data from the literature and highlight the antimicrobial qualities of the materials of choice, recommended in the treatment of gangrene in permanent teeth in children.Although MTA has demonstrated its antimicrobial qualities on the bacterial species studied here, it has not shown any superiority to calcium hydroxide in this regard, which remains the material with main indications for the induction of the apexification process.

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