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Evaluation of Antimicrobial Efficiency of Calcium Hydroxide in Combination with MTAD or Propylene Glycol against *Enterococcus faecalis*: An in vitro study

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ABSTRACT

Aim: To evaluate the antimicrobial efficiency of calcium hydroxide in combination with MTAD or propylene glycol against *Enterococcus faecalis*. ***Materials and Methods:*** The antimicrobial efficacy of intra-canal medicaments against *E.faecalis in vitro*, using 60 extracted single rooted teeth. The medicaments used in this study exerted antibacterial activity. The teeth were divided into 4 equal groups: Group I (calcium hydroxide + MTAD), Group II (calcium hydroxide + propylene glycol) and Group III (calcium hydroxide). Saline was taken as the negative control (Group IV). ***Results:*** Group I had the highest antimicrobial activity against *E.faecalis*. MTAD solution increases the antimicrobial efficiency of calcium hydroxide. Calcium hydroxide and calcium hydroxide + propylene glycol had nearly the same effect. There was a highly significant difference found between the three groups and the control group. ***Conclusions:*** MTAD combination with calcium hydroxide was the most effective against *E.faecalis* than calcium hydroxide + propylene glycol as intra-canal medicament.

Key words: Calcium hydroxide, colony counting, *E faecalis*; intra-canal medication, MTAD; propylene glycol.

Introduction

The main objectives of endodontic therapy are to eliminate bacteria from the root canal and prevent the growth of residual microorganisms. Antimicrobial agents are recommended for intra-canal medication to prevent the growth of microorganisms between appointments (Aravind *et al.*, 2006).
E. faecalis plays a major etiological factor in persistence of periradicular lesions after root canal treatment. Its prevalence in asymptomatic, persistent endodontic infection is about 77% (Vaghela *et al.*, 2011).

Calcium hydroxide has been widely used in endodontics. However, *E.faecalis* has been reported to be resistant to the antimicrobial effect of calcium hydroxide as a result of their ability to penetrate the dentinal tubules and adapt to the environmental changes (Evans *et al.*, 2002). Therefore, a search for a better alternative has led to various formulation of calcium hydroxide, using newer vehicles and antimicrobial agents such as MTAD and propylene glycol.

The strong antibacterial action of propylene glycol against the common microorganisms found in infected root canals and suggested its wider application in endodontics as a gentle vehicle for intra-canal medicaments. Its hygroscopic nature permits the absorption of water, which ensures a good sustained release of calcium hydroxide for long periods (Waltimo *et al.*, 1999).

Recently a new irrigant called MTAD (a mixture of tetracycline isomer, acetic acid and detergent) has been introduced as a final irrigant to be used after 1.3 % (NaOCl) (Torabinejad and Shabahangand, 2003). The results of some investigations have shown that MTAD can effectively remove the smear layer and abolish *E. faecalis* (Torabinejad *et al.*, 2003).

E.faecalis is a normal inhabitant of the oral cavity and is associated with different forms of periradicular disease. *E.faecalis* is highly resistant to calcium hydroxide. A combination of two medicaments may produce additive or synergistic effects (Vaghela *et al.*, 2011). Therefore, different vehicles have been added to calcium hydroxide in an attempt to enhance its antimicrobial activity against the tested microorganisms.

The persistence of bacteria in the root canal system often leads to failure of treatment. Microorganisms can colonize deep in the dentinal tubules that are not accessible to instruments and irrigation. Several studies have attempted to demonstrate the possible ways in which bacteria invade the dentinal tubules (Akpata and Blechman, 1982; Perez *et al.*., 1993). However, there are few reports on the efficacy of root canal medicaments against infected dentinal tubules under controlled conditions. *E. faecalis* was chosen as a test organism in this

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study because it is among the few facultative organisms associated with persistent apical periodontitis (Haapasalo *et al.*., 1983; Haapasalo and Orstavik, 1987) and it may be difficult to eliminate from root canals.

Many drugs, such as phenol, iodine solutions, formocresol and antibiotics, have been used for disinfection of the root canal. It is questionable whether sufficient amounts of commonly used medicaments can penetrate into tubules to achieve disinfection. The effectiveness of intra-canal medicaments based on phenol has been shown to decrease rapidly. Camphorated parachlorophenol has been found to be effective in inhibiting the growth of microorganisms in the root canal (Wantulok, 1972). This preparation releases chlorine slowly and is more germicidal. The camphor serves as a vehicle and reduces the irritating effect of pure parachlorophenol. It also has a prolonged antimicrobial effect (Grossman, 1988).

Material and Methods

Selection and standardization of root canals:

A total of 60 recently extracted sound permanent single-rooted human teeth having single root canal were collected. The teeth were cleaned from any calculus deposits and soft tissue debris by ultrasonic scalar, and then stored in distilled water containing 2% sodium azide (as an antimicrobial agent) until use.

Crowns of all the teeth were removed with a diamond disk and the root lengths were standardized to approximately 14 mm from the apex. For standardization of root canal diameter, Gates-Glidden drills sizes 4, 3 and 2 were used to prepare the root canal orifices. The apical thirds were prepared with K-file sizes 25, 30, 35 and 40 using the balanced force technique. The canals were irrigated with 1 ml of freshly prepared 1.3 % sodium hypochlorite solution after each file used. All the root canals received a flush of 5 ml of 17% EDTA (pH 7.2) for 1 min to remove the smear layer. These procedures were followed by final irrigation with 5 ml physiologic saline solution. Root canals were dried with sterile paper points. All the roots were sterilized by autoclaving (15 min at 121°C). The roots were kept in sterile water to avoid dehydration until use.

Infection of root canals

Under aseptic conditions, 10 µl of the previously prepared bacterial suspension was applied into each root canal using an automatic Eppendorf micropipette. Twenty-four hour colonies of pure culture of *E.faecalis* (ATCC 29212) grown on tryptone soya agar and M *enterococcus* agar was suspended in 5 ml of TSB for *E.faecalis* and incubated for 24 hours at 37°C. The root canals were transferred to a fresh broth containing microorganisms, every 3 day for 21 day.

After the incubation period, the sixty root canals were divided into four equal groups (n = 15)

Group I: Calcium hydroxide powder® assay 96% (Rolex Chemical Limited, Mumbai, India) + MTAD (Meta Dental New York, Elmhurst, USA).

Group II: Calcium hydroxide powder® assay 96% (Rolex Chemical Limited, Mumbai, India) + Propylene glycol (Hi Media)

Group III: Calcium hydroxide powder® assay 96% (Rolex Chemical Limited, Mumbai, India) mixed with 0.15 ml of the vehicle, i.e., distilled water] (Gangwar, 2011)

Group IV: Normal Saline (0.9%w/v; NaCl sigma) [control].

Each material was mixed according to manufacturer's instructions and applied into the root canal of each corresponding first, second and third groups. The forth group was irrigated only with saline.

Antimicrobial assessment

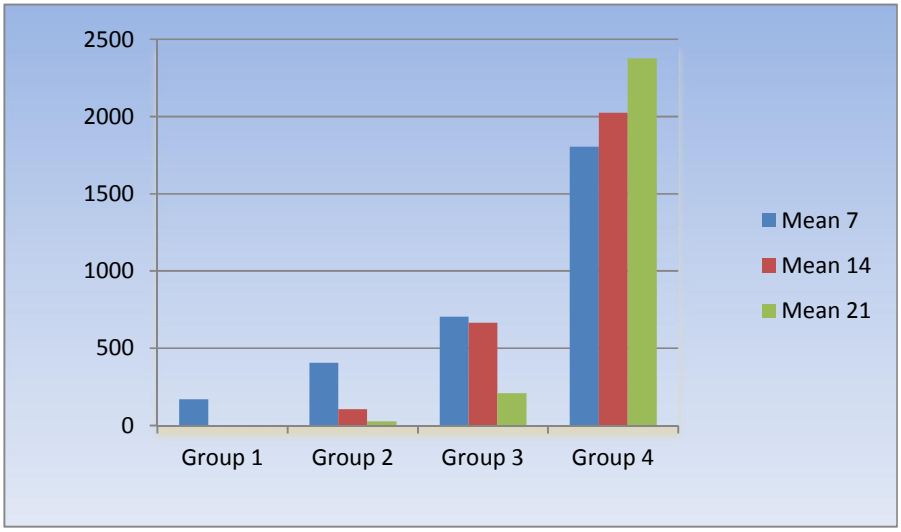
All the roots, after medication, were sealed above and below with paraffin wax and incubated in an aerobic environment, at 37° C. An antimicrobial assessment was performed at the end of the three intervals 7, 14 and 21 days. Dentin debris were harvested from each root canal using GG drills (Mani Inc., Japan) No. 4 and 5 ,respectively, collected in 1 ml of sterile broths, and incubated M-*Enterococcus* agar (Difico, England) plates on in aerobic environment at 37°C for 24 hours. After the incubation period, the content of each micro-centrifuge tube was evaluated by measuring the optical densities using the Multiskan Spectrum (Thermo Scientific) microplate spectrophotometer, at 620 nm and by using quantitative bacterial assessment serial dilutions of the resulting suspension were prepared (10)-1, (10)-2, (10)-3, (10)-4 and (10)-5 and aliquots of 0.1 ml on intervals of 7, 14, and 21 days were plated onto M-*Enterococcus* agar (M-*Enterococcus* agar) is a selective culture medium used to isolate *E.faecalis* and inhibit growth of other bacterial species, in which Triphenyltetrazolium chloride (TTC) is reduced inside the bacterial cells into insoluble formazan giving the characteristic red colonies. Sodium azid is added to suppress growth of gram negative organisms) at 37°C aerobically for 24h. The number of colony-forming units per milliliter (CFU/ml) that were available for counting found in the last three dilution, at which time colonies were counted.

Statistical analysis

The data were analyzed with one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test, to check the differences in inhibition of bacterial growth between the groups at different time intervals ($P< 0.01$).

Results

All the three medicaments used exerted antimicrobial activity. Group I (calcium hydroxide+ MTAD) had the highest antimicrobial activity at all time intervals. There was statistical significant difference between group I and all the other groups. Group II (calcium hydroxide+ Propylene glycol) and group III (calcium hydroxide) recorded inhibition of *E.faecalis* growth. There was statistically significant difference found between group II and group III. For the two medicaments the bacterial inhibition was significantly more on the 21 days (in which Group I and II was completely killed all bacteria). The inhibition of growth in all the groups was statistically significant in comparison to Group I [Figure and Table (1)]



	Mean		
	7	14	21
Group 1	169.7	0.933	0.667
Group 2	406.6	107.8	27.86
Group 3	704.6	665.1	210
Group 4	1806	2025	2375.2

($p<0.01$)

Fig. and Table 1: Comparison between the mean values of the four groups at 7, 14 and 21 days against *E.faecalis.*

Discussion

The results of a recent study show that hydroxyl ions derived from a calcium hydroxide intra-canal medicament diffuse in hours into the inner dentin but require 1 to 7 days to reach peak levels in the outer root dentin (Nerwich *et al*., 1993).

In another study, when compared with 2% iodine-potassium iodide solution, calcium hydroxide - dressed root canals yielded fewer culture reversals (Safavi *et al*., 1985, Sjögren *et al*., 1991) reported that calcium hydroxide efficiently eliminates bacteria which may survive after biomechanical instrumentation.

The findings of this study agreed with results of some investigators as (Torabinejad and Shabahang 2003). The higher effectiveness of calcium hydroxide against microorganisms in the dentinal tubules obtained from the combination of MTAD. Our control specimens were uniformly infected and yielded the highest number of microorganisms in the dentinal tubules. However, long-term in vivo investigations are needed for reliable testing of calcium hydroxide and MTAD. (Siqueira *et al.*, 1998).

The antimicrobial action of all the three medicaments increased on the 21 days. Propylene glycol was hygroscopic in nature, and therefore, there was a sustained release of hydroxyl ions from calcium hydroxide. This might be the reason for the increased antimicrobial activity of group II. Calcium hydroxide with MTAD vehicle had the diffusion of the paste within the tissues. This could be the reason of the better antimicrobial action of Group I.

Conclusion

From the results of this study it was concluded that:

- Calcium hydroxide with MTAD as a vehicle was an effective antibacterial intra-canal medicament against *E. faecalis*.
- The type of vehicle used would alter the antimicrobial property of calcium hydroxide as an intra-canal medicament.
- Addition of propylene glycol to calcium hydroxide as an intra-canal medicament made calcium hydroxide an effective antibacterial agent against *E. faecalis*.

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