COMPARISON OF THE ANTIMICROBIAL EFFECTS OF SIX DIFFERENT INTRACANAL MEDICAMENTS ON ENTEROCOCCUS FAECALIS.

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ABSTRACT

Aim: to investigate the antimicrobial effects of six different intracanal medicaments on Enterococcus faecalis.

Material & Methods: An agar well diffusion test was used to determine the efficacy of the experimental medicaments in removing E. faecalis (ATCC 29212). Medicaments were divided into 7 groups; calcium hydroxide (Ca(OH)2) with saline, Ca(OH)2 with anaesthetic solution, Ca(OH)2 with propylene glycol, commercially available premixed Ca(OH)2 paste, chlorhexidine gluconate gel, triple antibiotic paste (metronidazole, ciprofloxacin, doxicycline) with propylen glycol and talk powder with saline as negative control group. The diameters of the growth inhibition zones for each group were measured after 24 and 48 hours. Differences between groups were analysed using Kruskal-Wallis and Mann-Whitney U tests, and intragroup differences were analysed using Wilcoxon sign test.

Results: Diameter of the inhibition zone observed for the triple antibiotic paste was significantly larger ($p<0.01$) and the diameter of the inhibition zone observed for the chlorhexidine gluconate gel was significantly smaller in comparison to the other tested medicaments ($p<0.05$).

Conclusion: All of the tested medicaments were found to be effective on E. faecalis. However the results suggest that the triple antibiotic paste would be the preferred medicament against E. faecalis as it has the greatest antibacterial effect among the tested medicaments.

KEYWORDS : Agar well diffusion. Enterococcus faecalis. Triple antibiotic powder.
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INTRODUCTION

One of the most essential steps in root canal treatment is the elimination of the bacterias and their by-products. For this purpose, several irrigation solutions, intracanal dressing medicaments and root canal instruments have been used to achieve disinfection of the root canals. Because

of the complex anatomy of the root canals, the use of intracanal medicaments were advised to use in addition to chemo-mechanical preparation to improve bacterial elimination.[1]

Calcium hydroxide (Ca(OH)2) is the most commonly used intracanal dressing material.[2] Antibacterial effect of $Ca(OH)_2$ depends on maintenance of its high pH (12,5-12,8).[3] To improve and retain the pH effect, several paste vehicles such as aqueos, viscous and oily have been used. The vehicle plays a crucial role in determining the velocity of ionic dissociation. The solubilization and resorption of the paste also depend on it. If the viscosity is low, the ionic dissociation will be higher.[4]

Triple antibiotic paste (TAP) is the combination of metronidazole, ciprofloxacin and minocyclin. It has been developed for the disinfection of oral lesions consisting of pulpal and periapical pathologies.[5] When compared with $Ca(OH)_2$, TAP was prefered in large periapical lesions and retreatment cases because of its antimicrobial properties in in-vivo studies.[6] But, it has some disadvantages like discoloration and impossibility of removal from the root canals which prevent it from perfect intracanal dressing material.[7,8]

Enterococcus faecalis (E. faecalis) is a Gram-positive facultative anaerobic bacterium which is generally involved in endodontic treatment failures. It is the most common species isolated from secondary infections and root canal retreatments.[9,10] During the chemomechanical preparation of the canal, high-sodium alkaline environment can be obtained by both irrigation and inter-appointmet dressings. However *E. faecalis* can tolerate this high pH.[11] This situation was enlightened by Lleo et al in 1998.[12] They demonstrated that *E. faecalis* can enter viable but non-cultuvable phase (VBNC), in which they can survive under harsh conditions, remain alive[13] and continue to express virulence factors.[14] When optional conditions get gained, they can maintain their pathogenicity[12] and methabolic acticity.[15]

Taking cognizance of resistant structure of *E. faecalis,* it was aimed to evaluate the antimicrobial effects of TAP, chlorhexidin gluconate gel and several $Ca(OH)_2$ mixtures prepared with different vehicles on *E. faecalis* and compare each other in in-vitro conditions.

MATERIAL AND METHODS

An agar well diffusion test (AWDT) was used to determine the efficacy of the experimental medicaments in removing *E. faecalis* (ATCC 29212) (American Type Culture Collection, Manassas, VA, USA). Bacteria were subcultured from the stock culture on Brain Heart Infusion agar (BHI) plates incubated at 35°C for 24 h. In order to confirmation of the colonies, Gram staining was used. The cell suspensions were adjusted equivalent to 0.5 Mc Farland standards, adjusted to 1.5×10^8 CFU/mL^{-1} (CFU, colony forming units). Bacterial suspensions were dropped into the agar plates and inoculation was performed using sterile cotton swab sticks, rotating the plates around 60° in order to ensure the inoculum. Using a sterile agar puncher, wells of 6 mm width diameter were prepared in the agar plate.

Selected medicaments used in this study;

- Group 1: $Ca(OH)_2$ (Sultan Chemists Inc., Englewood, NJ, USA) with saline;
- Group 2: $Ca(OH)_2$ with anesthetic solution;
- Group 3: $Ca(OH)_2$ with propylene glycol;
- Group 4: Commercially available premixed $Ca(OH)_2$ paste, (Calasept, Scania Dental, Sweden);
- Group 5: Chlorhexidine gluconate gel, (Cerkamed Group, Nisko, Poland);
- Group 6: (TAP) (metronidazole, ciprofloxacin, doxycycline) with propylene glycol.

Talk powder with saline was chosen as negative control group. All calcium hydroxide groups were mixed at a ratio of 0.17g: 0.01 mL by mixing powder and solutions.[16] TAP was prepared according to the American Association of Endodontics (AAE) instructions at a ratio 1:1:1.

The experimental medicaments were added to the designed wells immediately after mixing up to the highest level of the agar. The plates were maintained at room temperature for 2 h to allow prediffusion of the materials. The diameters of the growth inhibition zones for each group were measured after 24 and 48 hours incubation (Figure 1). The diameters of inhibition zones

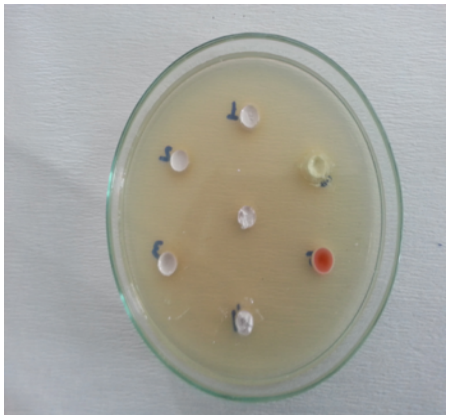

Figure 1a. Immediately placed medicaments.

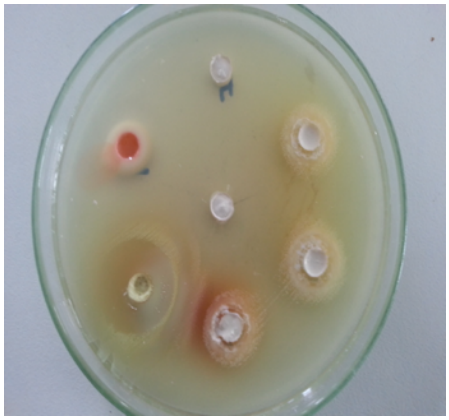

Figure 1b. 24h after incubation.

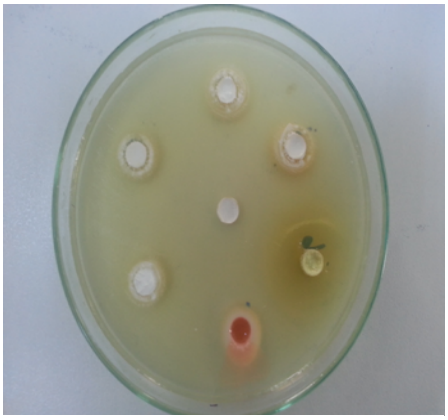

Figure 1c. 48h after incubation.

around the wells were measured and recorded for each medicaments. All measuremetns were performed by same operator. All the assays were repeated in 4 times. 4 times. All the assays were repeated in 4 times.

STATISTICAL ANALYSIS

Differences between groups were analysed using Kruskal-Wallis and Mann-Whitney U tests, and intragroup differences were analysed using Wilcoxon sign test.

RESULTS

Diameter of the inhibition zone observed for TAP was significantly larger ($p<0.01$) and the diameter of the inhibition zone observed for the chlorhexidine gluconate gel was significantly smaller in comparison to the other tested medicaments ($p<0.05$) (Figure 2). Increase in time resulted in greater antibacterial effect in all groups, especially in $Ca(OH)_2$ with propylene glycol and commercially available premixed $Ca(OH)_2$ paste ($p<0.01$, $p<0.05$).

DISCUSSION

The main etiological factor of pulpal and periapical diseases are microorganisms. In order to remove these microorganisms and their products, intracanal medicaments are still widely used. Agar disc diffusion test is one of the most fundamental antimicrobial susceptibility test. It is practical, simple, easy to interpreted by clinicians, qualitative and well-standardized.[17] However, it should be kept in mind that, agar disc diffusion is an *in-vitro* test conditions and several problems will affect the results such as ionig charge and conformation of the active ingredients, concentration and age of inoculum, volume and type of medium and the incubation conditions.[18] Nevertheless, this method is still in use at the present time. Estrela suggested that agar diffusion test is useful in the determination of the antimicrobial effect of $Ca(OH)_2$.[19] In the same study, 48 h were found to be sufficient to observe complete antimicrobial effect of $Ca(OH)_2$. In this study a waiting time of 48 h was applied to watch the inhibition zones, too.

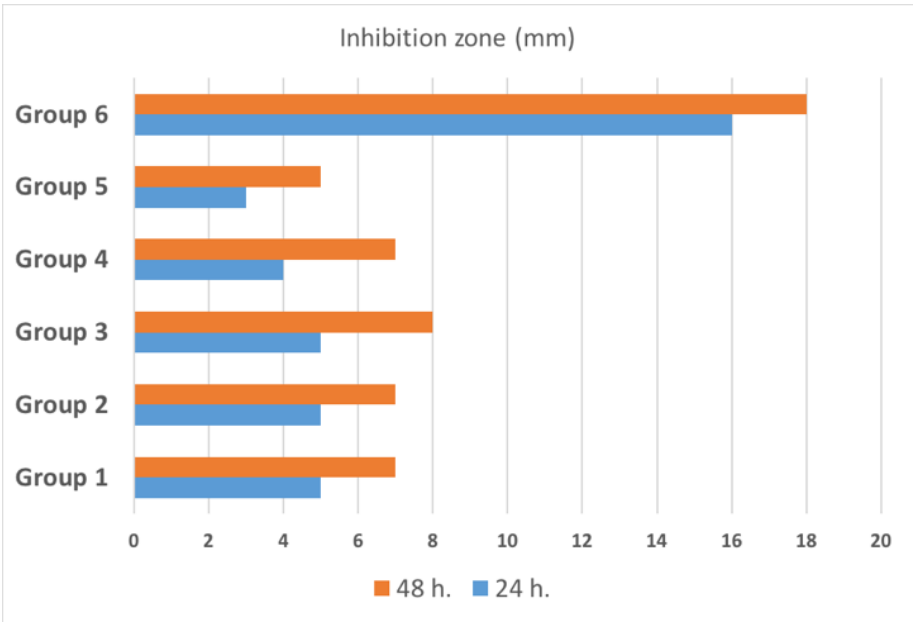

Figure 2. Inhibition zone.

When $Ca(OH)_2$ is mixed with a vehicle, an easily used and flowable paste forms. However, this paste should have some essential characteristics such as maintaining the high pH. The prophylene glycole, one of the viscous vehicles, was firstly used in endodontics as a vehicle in 1957.[20] Simon et al. indicated that it both helped handling the paste and made control the pH level and Ca^{+2} release possible.[21] They recomemended it as the best vehicle to prepare $Ca(OH)_2$ mixtures. Contrary, Behnen et al. showed that less viscous preparation of $Ca(OH)_2$ was more effective than the viscous ones in the elimination of *E. faecalis.*[22] In the present study, prophylene glycol, was used to observe its effect against aqueous solutions. When compared with saline (Group 1) and anesthesic (Group 2) vehicles, propylen glycol groups showed larger inhibition zone (Group 3

and 6). Furthermore, their antimicrobial effect also improved through time.

Mozayeni et al. reported that chlorhexidine gluconate gel was the most effective medicament against E. faecalis compared with $Ca(OH)_2$, TAP and nanosilver.[23] In the present study when considering both the diameter of inhibiton zone and increasing the antimicrobial efficacy in time, clorhexidine gluconate gel showed the poor properties which was statistically significant ($p<0.05$). This results may be related to the method that used to evaluate antimicrobial efficacy. Mozayeni et al. used extracted teeth and take samples from the root canals with paper points.[23] They also did not report their powder/liquid ratios and also used saline solution as a vehicle. In present study, agar plates were used. Because the extracted teeth were not used, the buffering effect of dentine may not be evaluated. Also, ratios were determined according to both AAE recommendations and previous studies. These may also effect the present study's results

E. faecalis gets through the growth, stationary and starvation phases. Starvation phase was shown to be the most resistant phase and living cells could not be eliminated by the medicaments totally.[24] Adl et al. evaluated the antimicrobial effect of both TAP and $Ca(OH)_2$ against *E. faecalis* in *in-vitro* conditions.[25] They reported that *E. faecalis* penetrated into 200 µm dentine depth and TAP was more effective than $Ca(OH)_2$ at the end of 7th day, which was in agreement with the study of Mozayeni et al.[23] The results of these previous studies are also correlated with the present research in which TAP resulted in the largest inhibition zone. However, TAP has some disadvantages, such as the inability to remove the antibiotics from the root canal and crown discoloration.[7,8] Thus, new medicines and antibiotic combinations should be developed.

CONCLUSION

All tested medicaments were found to be effective on E. faecalis. However, the results suggest that TAP would be the preferred medicament against E. faecalis as it has the greatest antibacterial effect among the tested medicaments. Increase in time may cause prolonged inhibition effect of Ca(OH)2 with propylene glycol and commercially available premixed Ca(OH)2 paste. Further studies should be performed to determine the best time intervals when applying these medicaments.

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