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ORIGINAL RESEARCH

Comparative Study of the Antimicrobial Effect of Three Irrigant Solutions (Chlorhexidine, Sodium Hypochlorite and Chlorhexidinated MUMS)

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ABSTRACT

Aim: To compare the antimicrobial effect of 2% chlorhexidine, 2.5% sodium hypochlorite and MUMS containing 2% chlorhexidine.

Materials and methods: All of the above irrigants were examined on *Enterococcus faecalis*, *Streptococcus mutans*, *Candida albicans*, *Lactobacillus casei* and *E.coli*. A total of 0.5 CC of each solution and 0.5 CC of McFarland solution bacterium were added to each examination tube. After 15, 30 and 45 minutes, colony count was performed for each tube. The difference in the number of bacteria indicated the effect taken by disinfectant material.

Results: MUMS containing chlorhexidine showed the antimicrobial properties just like chlorhexidine's effect against *E.coli*, *Streptococcus mutans*, *Candida albicans*, *Enterococcus faecalis* and *Lactobacillus casei* in preventing these entire microorganisms to incubate. Sodium hypochlorite was not effective against *Enterococcus faecalis* and *Candida albicans* incubated in 15, 30 and 45 minutes and *Enterococcus faecalis* in 15 minutes.

Conclusion: MUMS has antimicrobial properties similar to chlorhexidine.

Clinical significance: As MUMS containing chlorhexidine can transfer chlorhexidine through its own surfactant around apical area and it can open the dentinal tubules by its own chelator for more penetration of chlorhexidine, it may be a choice for canal irrigation.

Keywords: Antimicrobial activity, Irrigant, Chlorhexidine, Sodium hypochlorite, Microbial study.

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INTRODUCTION

The role of microorganisms in the pathogenesis of pulp and periradicular diseases has been established. [1,2] The purpose of root canal preparation is removing microorganisms from the canal by using biomechanical procedures accompanied with the use of antimicrobial agents. For decades, investigators have searched for antimicrobial agents that are more effective in debridement of the root canal system.[3-7]

An ideal irrigant should be an effective germicide and fungicide, be nonirritating to the periapical tissues, remain stable in solution, have a prolonged antimicrobial effect, be active in the presence of blood, serum and protein derivates of tissue, have low surface tension, not interfere with repair of periapical tissues, not stain tooth structure, be able to completely remove the smear layer, be able to disinfect the underlying dentin and its tubules and be relatively inexpensive. However, the common regimens in chemomechanical procedures using instrumentation and irrigation are not predictably effective in canal disinfection.[8,9]

Sodium hypochlorite (NaOCl) is the most common endodontic irrigant used. It presents strong antimicrobial activity and ability to dissolve necrotic pulpal tissue, so is usually chosen as a suitable canal irrigant. However, it is cytotoxic when it contacts periapical tissues.[10]

Chlorhexidine (CHX) is another antimicrobial agent that has been advocated for disinfection of the root canal system.[5] At low concentrations, it is bacteriostatic whereas at higher concentrations, it will cause the coagulation and precipitation of cytoplasm and therefore is bactericidal. [11]

MUMS is a newly-developed irrigant that contains chelating agent and surfactant. Its chelator is ethylene diamine tetraacetic acid (EDTA) and surfactants are polyoxyethylene sorbitan monooleate (Tween 80) and

sorbitan monooleate (Span 80). Tween 80 and Span 80 are generally regarded as nontoxic and nonirritating. MUMS changes the surface tension and so may be effective in delivering irrigant to the apical portion of the canal. The combination of CHX with MUMS may help in both antibacterial effects of CHX and smear layer removal of EDTA. On the other hand, it may block CHX from adhering to the root surface.

The purpose of this study was to compare the antimicrobial effect of 2% CHX, 2.5% NaOCl and MUMS containing 2% CHX on specific, most commonly associated microorganisms found within infected root canal over different time periods.

MATERIALS AND METHODS

Bacteria used in this study were *Streptococcus mutans* (ATCC 35668), *Lactobacillus casei* (ATCC393), *Candida albicans*, *E.coli* and *Enterococcus faecalis* isolated from patients. The bacteria inoculated in blood agar for 3 days at 35°C. After growing colonies, a smear was prepared from each petri and gram stained to confirm the presence of a single strain.

Four to five colonies of each bacterium from blood agar plates were solved in brain heart infusion (BHI) broth and incubated for 1 to 2 hours at 35°C until the bacteria reach the logarithmic growth phase and the number of microorganisms increase. Bacterial cells were resuspended in saline to give a final concentration of about 1.5×10^8 cells/ml, adjusted to 0.5 McFarland turbidity standards.

In one sterile glass tube, 0.5 ml of 2.5% NaOCl and 0.5 ml of *E.coli*'s broth were mixed and incubated after 15, 30 and 45 minutes in blood agar. This was performed for each microorganisms and each irrigants separately and repeated for 3 times. Colony forming units (CFU) were determined for each sample after 24 to 48 hours of incubation at 37°C.

Kolmogorov-Smirnov test was used for assessment of the normality of samples distribution. Man-Whitney test was used to compare the irrigants in three different times while critical level of significance was set at <0.05.

RESULTS

The effect of each irrigants on each microorganism in each period has been shown in Table 1. There were significant differences between 15 minutes (T1) and 30 minutes (T2) in CFU (T1 p = 0.000 and T2 p = 0.014).

Kolmogorov-Smirnov test showed that dates' distributions were not normal so Man-Whitney test was used to compare the irrigants in different times. This test showed that in the significance level of 5%, there was no significant difference between CHX and MUMS in T1 and T2 (p = 1.0) but the differences between MUMS containing CHX with NaOCl in T1 were significant (p = 0.029 and p = 0.029, respectively; Table 2).

In comparison of CFU of each irrigants in three different times, results showed that there was significant difference only in NaOCl group (p = 0.001). This test was not usable in two other groups of irrigants because the colony numbers were zero.

In NaOCl group, there were significant differences between T1 and T2 (p = 0.017) and between T1 and T3. In T1, *Enterococcus faecalis*, *Candida albicans* and *E.coli* could grow. In T2, *Enterococcus faecalis* was not obvious and the colony numbers of *Candida albicans* and *E.coli* reduced. In T3, the only colonies seen were *Candida albicans*.

DISCUSSION

Elimination of bacteria from the root canal system is essential for long-term success of endodontic treatment.[12] For this purpose, CHX has been shown to be effective against *Enterococcus faecalis in vitro*,[13] so it has been recommended for root canal disinfection.[5] Because of its inability to dissolve pulpal tissues, its use might be limited to a final rinse to enhance root canal disinfection. The pH of CHX is 5.5, and although the addition of citric acid and Tween 80 lowers its pH to 2.2, no significant difference in its ability to kill *Enterococcus faecalis* has been noted.[14] In the present study, there were no significant difference in antimicrobial effect of CHX and MUMS containing CHX.

NaOCl is the most commonly used antimicrobial irrigant in endodontics. This material has some advantages such as antimicrobial activity, tissue dissolving ability, lack of tooth discoloration and availability. A primary concern for the use of this chemical agent as a canal irrigant is its toxicity and potential for severe inflammatory response in the periradicular tissues.[15,16]

Table 1: The effect of each irrigant on microorganisms in 15, 30 and 45 minutes

Irrigant	*Streptococcus mutans*			*Lactobacillus casei*			*Candida albicans*			*Escherichia coli*			*Enterococcus faecalis*		
	15 mins	*30 mins*	*45 mins*	*15 mins*	*30 mins*	*45 mins*	*15 mins*	*30 mins*	*45 mins*	*15 mins*	*30 mins*	*45 mins*	*15 mins*	*30 mins*	*45 mins*
2% CHX	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
2.5% NaOCl	*	*	*	*	*	*	27,000	3,333	*	19,000	9,500	*	1,00,000	*	*
MUMS with CHX	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

*No growth

Table 2: Comparison of different irrigants in T1 and T2 with Mann-Whitney test

Irrigant		*Number*	*Mean*	*p-value*
T1	2% CHX	15	12	0.029
	2.5% NaOCL	15	19	
T2	2% CHX	15	13.5	0.217
	2.5% NaOCL	15	17.5	
T1	2% CHX	15	15.5	1.0
	MUMS with CHX	15	15.5	
T2	2% CHX	15	15.5	1.0
	MUMS with CHX	15	15.5	
T1	2.5% NaOCL	15	19	0.029
	MUMS with CHX	15	12	
T2	2.5% NaOCL	15	17.5	0.217
	MUMS with CHX	15	13.5	

The results of this study verified the effectiveness of CHX and MUMS containing CHX against bacteria and yeasts even in 15 minutes but in NaOCl group, no bacteria were killed in this time. In 30 minutes, no growth of *Enterococcus faecalis* observed with NaOCl. In the study of Vianna et al [17] 0.5 and 1% NaOCl required 30 and 20 minutes, respectively to completely kill *Enterococcus faecalis* and *Candida albicans*. Also, Radcliffe et al[18] reported that 0.5% NaOCl killed *Enterococcus faecalis* only after 30 minutes of incubation. Waltimo et al [19] showed that 0.5% NaOCl killed *Candida albicans* in 30 seconds. However, when diluted further to 0.05%, NaOCl did not kill *Candida albicans* even after 24 hours of incubation. Different results obtained in these studies can be related to the differences in the methods used and to the presence of confounding factors during the testing. The magnitude of the antimicrobial efficacy of a medicament can be influenced by the methodology, microbial characteristics in the biofilm, exposure time and concentration of the substance tested.[20,21]

Many root canal irrigants have good antimicrobial activity *in vitro* whereas *in vivo*, they often fail to completely eradicate all microbes. Several factors can reduce the effectiveness of them in *in vivo* conditions, such as poor penetration of the irrigants to the apical portion of the canal, localization of microorganism in the canal and dentinal tubules, insufficient exposure time, low concentration and presence of organic and inorganic compounds in the canal.[22] Also, despite the presence of a controversy regarding the effect of the smear layer on the quality of instrumentation and obturation, the smear layer itself may be infected and may protect the bacteria already present in dentinal tubules.[23] Because of these concerns, it may be necessary to remove the smear layer especially in infected root canals to allow penetration of disinfecting solution into the dentinal tubules in these teeth, so the irrigants can be able to penetrate all attribute of the canal.

To date, there is no single solution used in endodontics that simultaneously removes the smear layer and disinfects the entire root canal system except for MTAD.[24,25] MUMS in combination with CHX may offer another solution with these properties. One significant finding in the present study was the capacity of this solution to kill *Enterococcus faecalis* after 15 minutes. This ability was not observed with NaOCl.

A limitation of this *in vitro* study is that it does not account for the penetration ability of test irrigants in root canals. Other studies should be done to examine the efficacy of MUMS containing CHX as a final rinse in disinfecting experimentally infected human root canals.

CONCLUSION

Chlorhexidinated MUMS has antimicrobial properties similar to chlorhexidine.

CLINICAL SIGNIFICANCE

As MUMS containing CHX can transfer CHX through its own surfactant around apical area and it can open the dentinal tubules by its own chelator for more penetration of CHX, it may be a choice for canal irrigation.

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