Original Article

Synergistic Antimicrobial Efficacy of 2% Chlorhexidine and 3% Hydrogen Peroxide for Infected Root Canals of Extracted Human Teeth: An Ex-Vivo Study

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Abstract

Background and Aim: An endodontic irrigation solution with low toxicity and high antimicrobial activity is required in the process of endodontic treatment. Using a combination of intracanal irrigants is one solution to achieve this goal. The aim of this study was to evaluate the synergistic antimicrobial efficacy of chlorhexidine and hydrogen peroxide against bacteria in the infected extracted human root canals.

Materials and Methods: Forty-two teeth with periapical lesions were chosen for this study. The teeth were extracted and preserved in normal saline at 37°C for less than 24 hours. Canals were prepared with nickel titanium rotary files (S1 to F3) and irrigated with 10cc of the respective irrigants (group 1:14 teeth irrigated with 3% hydrogen peroxide; group 2: 14 teeth irrigated with 2% chlorhexidine and group 3: 14 teeth irrigated with a combination of 3% hydrogen peroxide and 2% chlorhexidine). Samples were obtained with paper cones before and after root canal preparation (S1, S2) and transferred to a microbiology lab for colony counting. Data were analyzed using the Kruskal-Wallis and Mann-Whitney tests.

Results: The results showed that all the understudy irrigants significantly decreased the bacterial colony count. A combination of chlorhexidine and hydrogen peroxide was significantly more effective than hydrogen peroxide.

Conclusion: Chlorhexidine is an effective irrigant with high antimicrobial activity but its antimicrobial efficacy does not significantly increase in combination with hydrogen peroxide.

Key Words: Chlorhexidine, Hydrogen peroxide, Synergistic effect, Root canal irrigant, Antibacterial effect

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Introduction

Bacteria remaining in the RCS are the main cause of failure of endodontic treatments [1]. The ideal goal in endodontic therapy is to obtain a root canal free from living microorganisms and their byproducts. To achieve this goal, mechanical debridement is often performed in conjunction with the use of canal irrigating solutions.

A suitable root canal irrigant must have adequate antimicrobial activity and be able to dissolve organic tissues to enhance debridement of the RCS. Also, it must be safe and non-toxic for periapical tissues [2].

Root canal cleaning and shaping is the most important step in endodontic therapy. Mechanical preparation alone cannot result in complete elimination of bacteria from the RCS due to its complexity and presence of hard-to-reach areas [2, 3]. Thus, irrigants with strong antibacterial properties are required to be used in conjunction

with mechanical preparation in order to eliminate microorganisms and dissolve tissue residues. The main characteristics of endodontic irrigants include: antimicrobial activity, dissolution of organic tissues, enhancing the debridement of RCS and being non-toxic to periapical tissues [4].

Several irrigants have been introduced for root canal irrigation but none of them is ideal for this purpose [3].

Sodium hypochlorite has been used for canal irrigation for several decades. It is an efficient antimicrobial agent and well dissolves the organic residues [4, 5]. However, it has some drawbacks including unacceptable odor and taste, high cytotoxicity, and stimulation of the periradicular tissues (if leaks out of the apex) [6, 7]. As the result, some other irrigants have been suggested for use as an alternative to sodium hypochlorite, and CHX is one of them [8].

Chlorhexidine digluconate is a synthetic bis-biguanide used as a broad-spectrum antimicrobial agent in dentistry for years. Due to its cationic nature, it is capable of forming an electrostatic bond to negatively-charged bacterial surfaces. As the result, it damages the outer layer of bacterial cell wall and increases its permeability. Depending on its concentration, it can have bacteriostatic or bactericidal effects. It has excellent antimicrobial property and acceptable taste and odor. Its insignificant cytotoxicity and acceptable substantivity are among other favorable characteristics of CHX [9-12].

Hydrogen peroxide is also used as an intracanal irrigant in 3%-5% concentrations [11, 13]. Its antimicrobial effect is due to hydroxyl radicals and it is effective against bacteria, fungi, yeasts and spores. Hydroxyl radicals can attach to membrane lipids, DNA and cell organelles and cause death of the microorganisms [14]. The synergistic antibacterial effect of CHX and H2O2 was recently proposed.

Heling and Chandler evaluated the antimicrobial efficacy of a combination of CHX and H2O2 in bovine dentinal blocks inoculated with E. faecalis and reported that this combination was more effective than the application of each of them alone [12].

Stinberg et al. used a combination of several bacteria including E. faecalis in a culture medium rich in peptide and reported that the combination of CHX and H2O2 eliminated E. faecalis in the culture medium in a concentration lower than that of each of them used alone [13].

Shahriari et al, in their study on dentinal blocks of extracted human teeth concluded that H2O2 had no effect on substantivity of CHX [14].

In the aforementioned studies on the synergistic effect of CHX and H2O2 on human and bovine dentinal blocks, only one specific strain was evaluated since the microbial flora of the canal had been eliminated. Thus, conduction of a study on extracted human teeth with infected root canals seemed necessary due to better simulation of clinical setting because of the presence of microbial biofilm containing the majority of intracanal microorganisms as well as anatomical complexity of the RCS enabling application of mechanical debridement and chemical irrigation. Moreover, in studies on dentinal blocks, the possibility of false positive results is high due to the high risk of external contamination of specimens [3]. Thus, in-vivo and ex-vivo studies are required to be performed on human extracted teeth with infected canals. The current study aimed to assess the efficacy of a combination of 2% CHX and 3% H2O2 as an intracanal irrigant for extracted human teeth with necrotic pulp and apical periodontitis.

Materials and Methods

This in-vitro study was conducted on 42 human, extracted, single-canal teeth. Lack of canal calcification and anatomical abnormality was ensured radiographically. The teeth had clear periapical lesion before extraction and had to be extracted due to extensive, non-restorable caries. The teeth were painless with no abscess or sinus tract, responded negatively to pulp vitality tests and were not sensitive to percussion or palpation. Teeth with previous root canal therapy, patients with history of antibiotic therapy within the past three months and those with advanced periodontal disease were excluded. Immediately after extraction, the teeth were immersed in saline solution at 37°C for 24 hours. Root canal of single-canal teeth, distal canal of mandibular molars and palatal canal of maxillary molars were used. The external surface of teeth was disinfected

with 30% H2O2 followed by 2.5% sodium hypochlorite before sampling. Tooth crowns were cut at the cement enamel junction by a sterile diamond bur. The external surface of teeth was disinfected again as explained earlier. Next, 5% thiosulfate was used to neutralize hypochlorite [15]. Primary sampling of the root canals (S1) was done by a #25 paper point. To prevent deformation of paper point during sampling, preflaring was done using a #15 sterile hand file (Mani, Japan) to a length 1 mm short of the working length. If the canal was dry, it was filled with normal saline up to the orifice. Primary samples (S1) were aseptically transferred to thioglycolate culture medium (Merck, Germany). The coronal two-third of the canal was flared by Gates Glidden drills and the apical segment was prepared using S1-F3 ProTaper nickel titanium rotary files (Maillefer, Switzerland). The teeth were randomly divided into three groups of 14. In group 1, specimens were disinfected with 3% H2O2, in group 2, with 2% CHX and in group 3 with a combination of equal amounts of the H2O2 and CHX. After each time of using rotary files, the root canals in each group were rinsed with 2cc of the respective solution with a total volume of 10cc by a 5cc syringe and 27 gauge needle. All teeth were then rinsed with 5cc of distilled water to wash out the irrigant from the RCS. It should be mentioned that CHX was prepared by diluting 20% solution (Sigma, Germany) and H2O2 was prepared by diluting 30% solution (Merck, Germany). Based on previous studies, the synergistic effect of 3% H2O2 and 2% CHX was evaluated [12-14].

Using two #35 and #40 sterile paper points, second samples (S2) were obtained from the root canals. Paper points were placed in the root canals for one minute to absorb moisture and the specimens were then transferred to thioglycolate culture medium and sent to microbiology lab in less than 60 minutes. In the lab, the specimens were vortexed for 30 seconds and 10 times serial dilutions were prepared; 100µl of the prepared dilutions was spread over Brucella agar plates (Merck, Germany) containing 5% defibrinated sheep blood, 5 mg/L hemin (Sigma, Germany) and 1mg/L menadione (Sigma, Germany). The plates were then incubated in an anaerobic jar (Anoxomat, Netherlands) at 37°C for 7 days. After completion of incubation, colony forming units (CFU) were counted in 1/100 and 1/1000 dilutions and number of CFU per mL was determined based on dilution and volume.

The results of the three groups were compared and data were analyzed using the Kruskal Wallis test, Dunn test and SPSS 15. The Wilcoxon Signed Rank test was applied to compare the bacterial count of secondary and primary samples in each group. The Kruskal Wallis test was used for the comparison of three groups in terms of relative changes in CFUs. Dunn test was used for pairwise comparison of groups. Chi- square test was applied for comparison of groups in terms of yielding colony-free specimens. Fisher's exact test was also used for pairwise comparison of groups.

Results

Table 1 summarizes the mean percentage of bacterial count reduction (CFU_{S2}-CFU_{s1}), standard deviation (SD) and P values of different groups**.** The results showed that S1 samples of all canals were infected with bacteria. The Kruskal Wallis and the Wilcoxon tests showed that all S2 samples had significantly lower bacterial count compared to S1 samples (p=0.029) (Table 1).

Table 2 shows the mean and SD of relative changes in CFUs in groups and the P values for pairwise comparison of groups.

The Kruskal Wallis test indicated a significant difference in change in CFUs in the three groups (p=0.029). Pairwise comparison of the three groups by Dunn test showed that the relative magnitude of reduction in bacterial count in the CHX+H2O2 group was significantly greater than that in the H2O2 group alone (p=0.039-0.024). However, no significant difference was found in this regard between the H2O2+CHX and the CHX group (p=0.194-0.371) or CHX and H2O2 groups (p=0.206-0.720).

As seen in Table 3, after irrigation of root canals with H2O2, 7 specimens had negative bacterial culture. This number was 9 in the CHX group and 13 in the combined group. The chi-square test indicated a significant difference among groups in yielding colony-free specimens (p=0.044). Pairwise comparison of groups with Fisher's exact test showed that the combined group (group 3) had significantly higher number of colony-free samples than group 1 (p=0.033). But, the difference in this

Table 1. Comparison of CFUs before and after root canal preparation

Groups	S1			S2			P value
	Mean	SD	Median	Mean	SD	Median	
H2O2	81.07	43.28	75.000	2.79	4.23	1.50	0.001
CHX	210.00	130.69	180.00	1.86	3.57	0.00	0.001
Mixed	56.86	56.63	33.00	0.21	0.80	0.00	0.001

*Mean CFU before and after root canal preparation
*Wilcoxon Signed Rank test

Table 2. Comparison of changes in CFU (CFU2-CFU1/CFU1) in the three groups

-	Group	Number	Mean	SD
1	H2O2	14	-%96.9	0.05
2	CHX	14	-%99.1	0.01
3	H2O2+ CHX	14	-%99.8	0.005

*Mean percentage of bacterial count reduction (CFU_{s2}-CFU_{s1})
*P value of group 1 compared to group 2:0.720-0.306
*P value of group 2 compared to group 3:0.371-0.194
*P value of group 1 compared to group 3:0.024-0.039

regard between groups 1 and 2 (p=0.704) or 2 and 3 (p=0.165) was not significant.

Discussion

Researchers have always been in search for a suitable root canal irrigant with ideal characteristics like high antimicrobial activity and low cytotoxicity. Many investigators have used a combination of root canal irrigants to maximize the positive effects and minimize the side effects [12-14, 16-18]. The synergistic effect of CHX and H2O2 has been evaluated in several in-vitro studies [12-14]. Our study was methodologically similar to those by White and Jeansonne [4] and also Delang et al, [15] and we tried our best to simulate in-vivo conditions. Our results showed that CHX alone and in combination with H2O2 was capable of elimination of root canal bacteria and no significant difference was noted in this regard between the mentioned two groups (p=0.194). Our results in this respect were not in line with those of Heling and Chandler and Stinberg et al. In the current study, significantly greater reduction in bacterial count was observed in the combined group compared to H2O2 alone (p=0.039); but, this reduction was not significantly different from that in the CHX group alone (p=0.194). Such difference in results may be due to different methodology of studies. The previous studies had an in-vitro design and mainly evaluated only one type of microorganism. Poly-microbial infection of the RCS, presence of organic compounds, anatomical complexity of the RCS and role of mechanical debridement were not evaluated in the previous studies. Jeansonne and White [4] and Ercan et al [19]. Reported that after using 2% CHX as root canal irrigant, 70% of specimens were colony-free; which is close to 64.3% rate observed in the current study. They concluded that CHX and sodium hypochlorite had similar antibacterial efficacy.

Vijaykumar et al, [17] in 2010 evaluated and compared the efficacy of several irrigants including 3% H2O2 against E. faecalis. In their study, H2O2 yielded 33.3% colony-free specimens. In the current study, H2O2 resulted in elimination of 50% of colonies; this difference in results may be due to different methodology and type of microbial testing. In their study, only one type of microorganism was evaluated while in the current study, various microorganisms present in the RCS were evaluated. Kuruvilla et al [16].

Evaluated the efficacy of hypochlorite in combination with CHX in elimination of root canal bacteria in comparison with the application of each of them alone.

Table 3. Comparison of colony-free groups in the three groups

-	Group	Number of colony-free specimens	Percentage of colony-free specimens
1	H2O2	7	%50
2	CHX	9	%64.3
3	H2O2+ CHX	13	%92.9

*P value of group 1 compared to group 2:0.704
*P value of group 2 compared to group 3:0.165
*P value of group 1 compared to group 3:0.033

They reported that CHX alone caused 70% reduction in bacterial count after irrigation while this rate was 59.4% for hypochlorite and 84.6% in combined group.

Reduction in bacterial count in the mixed group was significantly higher than that in the hypochlorite group alone; but, the reduction in CHX and combined groups was not significantly different.

Considering all the above, we may conclude that CHX alone can be used as an effective antimicrobial solution since it is capable of causing significant reduction in bacterial count.

Although many in vitro studies have reported the synergistic antimicrobial effect of CHX and H2O2, by better simulating the clinical setting and in vivo conditions, this synergistic effect becomes less significant probably due to complex root canal anatomy and poly-microbial nature of infections in the RCS.

The current study showed that combination of H2O2 and CHX eliminated bacterial colonies in 92.9% of specimens. CHX caused 64.3% reduction in microbial count and the difference in this regard between the CHX and the combined group was not significant. Thus, the authors do not recommend using CHX in combination with H2O2.

Conclusion

CHX alone has strong antimicrobial properties and is suitable for decontamination of the RCS. Its efficacy does not significantly increase when combined with H2O2. Combination of 2% CHX and 3% H2O2 following mechanical root canal preparation does not have a superior antibacterial efficacy in comparison with 2% CHX alone. However, this combination has superior antimicrobial efficacy compared to that of H2O2.

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