Original Article

Antimicrobial Efficacy of 2.5% Sodium Hypochlorite, 2% Chlorhexidine, and 1.5% Hydrogen Peroxide on Enterococcus Faecalis in Pulpectomy of Necrotic Primary Teeth

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Abstract

Introduction: The success of the endodontic treatment is closely associated with eliminating endodontic microbiota especially bacteria like Enterococcus Faecalis (E. Faecalis). Irrigation solutions are suggested for this purpose but there are contraries regarding irrigations and their concentrations. This study aimed to compare antibacterial efficacy of irrigations including 2.5% Sodium hypochlorite (NaOCl), 2% Chlorhexidine (CHX), and 1.5% Hydrogen peroxide (H2O2). **Methods:** Fifty deciduous human extracted teeth were divided into 3 groups of 15 teeth, 2.5% NaOCl, 2% CHX, 1.5% H2O2, and 5 teeth in the negative control group. Later, root canals were inoculated by E. Faecalis. After cleaning and shaping, we irrigated the root canals of the teeth in each group with NaOCl, CHX, and H2O2. Samples were obtained again and sent for microbiological evaluation. Wilcoxon signed-rank test, Paired sample T-test, and Kruskal–Wallis were used to analyze data. **Results:** All 3 groups showed significant bacterial reduction ($P<0.05$). NaOCl and CHX showed no significant difference ($P=0.415$). But the reduction of these 2 groups was higher than H2O2 ($P<0.001$ for each). **Conclusions:** 2.5% NaOCl and 2% Chlorhexidine showed considerable efficacy against E. Faecalis while 1.5% Hydrogen peroxide was not able to eradicate all of E. Faecalis colonies. Hence, NaOCl and CHX solutions can be used for decontamination of infected root canals.

Keywords: Sodium hypochlorite, Chlorhexidine, Hydrogen peroxide, Primary teeth, Pulpectomy, Enterococcus Faecalis

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Introduction

The preservation of primary teeth is one of the most important goals of pediatric dentistry to maintain harmonious growth and development of arch length and occlusal balance. This will provide optimal function for swallowing, chewing, speech, and aesthetics(1). Pulpectomy is a treatment performed for carious primary

teeth with necrotic or irreversible pulpitis. The purpose of a pulpectomy is to eradicate microorganisms from root canal system by mechanical debridement and chemical irrigation. The main reason for pulpectomy failure is remaining bacteria such as Enterococcus faecalis, Streptococcus mutans, and Candida albicans in the root canal system(2). Enterococcus faecalis (E. faecalis) is one of the major bacteria in recurrent root canal infection. This is one of the most resistant species to treatment and a possible reason for failure of root canal treatment(3). Some characteristics of this microorganism that cause resistance against chemomechanical irrigation are deep penetration into dentinal tubules(4), high pH tolerance(5), surviving in food deprivation(6), surviving in root canal without support of other bacteria(7) and adhesion to collagen fibers(8).

Currently, there is no agreement among pediatric dentists about the best anti-bacterial irrigation in endodontic treatment of primary teeth. This is probably because of limited on this topic(9). Contemporary literature has numerous reports on efficacy of 5.25% hypochlorite(10,11), however damage to permanent teeth follicles, peripheral tissue, and oral mucosa have been reported during inappropriate use of 5.25% NaOCl in pediatric endodontic treatments(12). It has been shown that actions and toxicity of NaOCl are dose-dependent, therefore 2.5% NaOCl could be less toxic than the 5.25% concentration. However, there are some doubts about its antimicrobial effectiveness. According to some studies, 2.5% NaOCl is considered to be efficient(13,14), while others were on the contrary of these findings (15,16). Another frequently used irrigation is chlorhexidine gluconate. Though it has demonstrated a significant antibacterial effect, its inability to dissolve necrotic tissue has raised some doubts about it(17). Although hydrogen peroxide exhibits a broad spectrum of action against bacteria, viruses, yeasts, and bacterial spores, some studies have suggested that it has less antimicrobial activity than NaOCl as a root canal irrigation solution(18).

Due to importance of employing a harmless irrigation in pulpectomy of primary dentition and controversies regarding this matter, the main purpose of this in-vitro study was to evaluate 2.5% sodium hypochlorite, 2% chlorhexidine, and 1.5% hydrogen peroxide efficacy on E. Faecalis.

Material and methods

Specimen preparation

Our study was done according to the guidelines of the Declaration of Helsinki and also approved by the ethical committee of Mashhad University of Medical Sciences, Mashhad, Iran (no:910880). For this experimental in-vitro study, 50 anterior primary teeth that had been extracted due to pulpal necrosis and periapical lesions were collected. These teeth had intact roots or less than 2/3 of physiological root resorption with no previous root canal treatment. The sampling method was non-probability and purpose-based and the sample size for each group was calculated as n = 15, based on an alpha significance level of 0.05 and a beta of 0.2, according to the data obtained from a previous study(19). Hence, having 3 groups and 5 negative control teeth the total sample size of this study was 50 primary teeth. After extraction, external surfaces of the roots were debrided using a curette. Then all teeth were disinfected in 0.5% NaOCl (Chloraxid, Cerkamed, Poland) for 24 hours and then in 0.9% saline at room temperature until tests were performed. The crowns of the teeth were cut at CEJ by long cylindrical diamond bur (no 883, Jota, Switzerland and a high-speed handpiece. So, length of the roots became 10-12 millimeters. Then we used appropriate Hedstrom files (Mani Inc, Tochigi, Japan) to remove pulpal remnants and debris from canals. The canals were then prepared by passive step back method using K-files (Mani Inc, Tochigi, Japan). We also used Cyanoacrylate adhesive (Incredible DROP, Iran) for sealing terminals to prevent microorganism diffusion and allow handling of teeth during the experiment. The smear layer of each sample was removed in an ultrasonic bath with 17% Ethylenediamine tetraacetic acid (EDTA) (Aria Dent, Asia Shimi Teb, Iran) and then 5.25% NaOCl (Chloraxid 5.25%, Cerkamed, Poland) (by Yamada et al. Suggested method(20).

Finally, samples were rinsed with sterile distilled water for 10 minutes. Teeth samples were sterilized using an autoclave.

Contamination of root canals

We contaminated teeth by injection of a pure culture of E. faecalis (ATCC 29212) suspension in brain heart infusion (BHI) broth with 0.5 Mc Farland concentration (1.5x108 bacteria per ml) into the canals with an insulin syringe. Five samples received sterile BHI broth and served as a negative control group to confirm sterilization conditions. Then each tooth was transferred to 1.5 ml microtubes under aseptic condition. All micro tubes were placed in an incubator at 37°C for 48 hours.

Disinfection

After this period, each sample was placed in sterile normal saline 3 times and each time 30 seconds to remove excess broth and bacteria on the outer surface of the tooth. Then a specific volume of physiology serum was injected into canals using insulin syringes and carried to sterile microtubes for counting bacteria in BA and BEA cultures and CFU determination (Colony Forming Unit)by standard plate count method(21).

The samples were randomly separated into 3 experimental groups (n = 15). The canal of each one was filled with 5 ml of the following solutions and irrigated for 5 min: 1.5% hydrogen peroxide (1.5% Hydrogen Peroxide Solution, Dr. Mirhadizadeh Lab, Mashhad, Iran), 2% CHX (GLUCO-CHEX 2%, Cerkamed, Poland CERKAMED), and 2.5% NaOCl (Chloraxid, Cerkamed, Poland) respectively. As a negative control, one group was not irrigated (n = 5). Chlorhexidine gluconate was used as a positive control. After the disinfection procedures, the root canals in all samples were washed 3 times by using 1 ml of sterile saline solution. We counted bacteria for the second time and reported it as CFU and then compared it with the preliminary results.

Statistical Analysis

All statistical analyses were performed using the SPSS for Windows TM, version 16 software package (SPSS Inc., Chicago, IL, USA). Data were expressed as means ± SD for parameters with a normal distribution. Shapiro-Wilk test was used to assess normality.

Group comparisons were performed using student's T-test and Mann-Whitney test, as appropriate. Paired sample T-test and repeated measures ANOVA were used for analyzing parametric variables. The significance level was set at $P < 0.05$.

Results

We analyzed 50 teeth for presence of E. Faecalis (Table I). All 3 irrigations demonstrated significant bacterial reduction ($P<0.05$). We had no reduction in our negative control group. Two of the irrigations (NaOCl and CHX) were better against E. faecalis and eradicated all remaining bacteria. The largest mean reduction was demonstrated in the group irrigated with 2.5% NaOCl followed by the group rinsed with 2% CHX (positive control) and the group washed with 1.5% Hydrogen peroxide. The bacterial reduction in groups irrigated with NaOCl and CHX was significantly higher than the group in which hydrogen peroxide was used ($P<0.001$). No substantial difference was found between NaOCl and CHX in bacterial reduction ($P=0.415$).

Table I: Results of cultures before and after irrigation of the root canals contaminated with *Enterococcus faecalis*

Irrigant	Before irrigation (CFU)	After irrigation (CFU)	P value*	Reduction	Kruskal-Wallis (Reduction)
2.5% NaOCl	100333±3994	0	P=0.001*	100333±399	
2% CHX (positive control)	99333±4577	0	P=0.001	99333±4577Ψ	P<0.001
1.5% H_2O_2	99333±8208	19933±1486	P<0.001**	79400±7048Ψ	

* Wilcoxon signed-rank test (significant at p<0.05); **Paired sample-T test (significant at p<0.05); +: Mann-Whitney post-hoc showed significant difference (P<0.001); Ψ: Mann-Whitney post-hoc showed significant difference (P<0.001); CHX: Chlorhexidine; CFU: Colony Forming unit

Discussion

The success of endodontic therapy is associated with the control of intracanal micro-organisms. E. faecalis is one of the etiologic factors for the failure of endodontic treatments and it's related to peri-apical lesions refractory to endodontic treatments (22,23). It has been shown that it is difficult to eliminate this bacteria; consequently, the use of irrigants becomes essential as it can enhance mechanical debridement. Also, it is the only way to clean some parts of the root canal wall that are not touched with the aid of mechanical instrumentation(24). Several studies have compared The characteristics of

currently used irrigants (25,26); However, most of these studies were conducted on permanent teeth (27).

Goals of irrigation are: Decrease of intra-radicular microorganisms and inactivating their endotoxins, dissolving necrotic or vital pulp tissue, Lubrication of canal walls and instruments, and Removal of smear layer. Various products such as sodium hypochlorite, chlorhexidine, and hydrogen peroxide are used for this purpose(28).

One of the irrigants we used in this study was sodium hypochlorite (NaOCl). NaOCl is a commonly used endodontic irrigant(29-31). It has antimicrobial activity and the ability to dissolve residues of necrotic tissue, pulpal remnants, and collagen(32). The recommended concentration for NaOCl in many studies is 5.25%. A study conducted by Bhasin et al.(33) showed that 5.25% NaOCl can considerably reduce E. faecalis load, but it has been proven that this concentration can damage permanent teeth follicles if it is used in deciduous teeth(12). Therefore, irrigation during endodontic treatment of deciduous teeth is a bit different due to root resorption, open apex, and presence of permanent tooth bud. It is always necessary to pay attention to stimulate the periapical tissues and the inflammatory reaction because of irrigant functions and its adverse effect on the permanent tooth bud.

in some other previous studies, it is mentioned that 5.25% sodium hypochlorite (NaOCl) has acceptable antimicrobial activity(22,34), but the cytotoxicity of this solution on periapical tissues has been reported(16). A problem with sodium hypochlorite is an injection of that beyond the apical foramen and tissue necrosis. It additionally has cytotoxic characteristics and cannot eliminate the smear layer made during instrumentation, which led to the look for new options for solutions with the same antimicrobial activity but lower toxicity(32). Negative findings of toxicity, recommended dilution of 5.25% NaOCl to lower concentrations(35).

We used 2.5% NaOCl in our study. Our findings suggest that this concentration is appropriate and can eliminate all bacteria from root canals. Some studies stated that this concentration is acceptable enough(14,22), but some others stated the opposite results(16,36) . In our study, after using 2.5% sodium hypochlorite, the growth rate of bacteria was zero. Siqueira et al.(37) proved a result similar to ours while in the study of Buck et al.(38) the rate of E. faecalis decreased but not eliminated completely. The reason for the difference between the results of our study and this study could be the difference in the concentration of NaOCl and the reduction of its antimicrobial effect.

In our study, another irrigant was chlorhexidine gluconate (CHX). According to our results, CHX was effective against E. faecalis at concentrations of %2. Similar to our study, Singh et al. (26) stated that NaOCl and CHX had an antimicrobial effect on E. faecalis and there was increased antimicrobial efficacy with increasing concentrations.

CHX is a cationic biguanide. It can eliminate both gram (--) and gram (++) bacteria as well as yeasts and it is effective against strains resistant to Ca(OH)2 that is a medicament dressing the canal. A concentration of 2% chlorhexidine is more effective than lower concentrations. CHX at low concentration, act by adsorbing onto the cell wall of a microorganism and leakage of intracellular constituents, especially potassium and phosphorus, resulting in a bacteriostatic effect. It also at a high concentration, has a bactericidal effect because of deposition and/or coagulation of intracellular components, likely caused by cross-linking proteins (39).

The results of the present study showed that CHX removes 100% of E. faecalis bacteria from the root canal. Olivia et al. (40) Obtained similar results and showed that a concentration of 2% chlorhexidine removed E. faecalis from the root canal. The effect of chlorhexidine on E. faecalis is due to the reaction between the positive charge of chlorhexidine molecules with the negative charge of phosphate groups in the bacterial cell wall which causes a loss of bacterial cell somatic balance and lysis. Menezes et al. (15) showed that the use of 2% CHX significantly reduced E. faecalis in the root canal but could not completely eliminate the bacteria. This discrepancy may be due to differences in the method of microbial sample collection.

We found that CHX is as effective as NaOCl. In the results of our study, there was no significant difference between 2.5% NaOCl and %2 CHX. Similar to our findings, Ahangari et al.(41) reported no significant difference between antimicrobial efficacy of 2,5% NaOCl, MTAD, and %2 Chlorhexidine gluconate against E. faecalis in the root canals of extracted and single-rooted permanent teeth of humans. Vianna et al. (42) stated that CHX is more effective against E. faecalis compared NaOCl. However, it can't dissolve necrotic pulp tissue . Jeansonne et al.(43) found that 2% CHX had similar antibacterial effectiveness to 5.25% NaOCl and also has lower toxicity, therefore we can use it for patients that have an allergy to NaOCl .

The third irrigant in our study was hydrogen peroxide (H2O2). A clear and odorless liquid uses in dentistry with different concentrations from 1% to 30%. It is active against bacteria, bacterial spores, viruses, and yeasts via

the production of hydroxyl free radicals which attack proteins and DNA. It is an unstable compound that decomposes through heat and light. H2O2 acts with the aid of freeing nascent oxygen upon which coming in touch with tissue enzymes produces a bactericidal impact by way of interfering with bacterial metabolism(24).

We found that 1.5% H2O2 reduced the amount of E. faecalis but didn't completely remove it from the root canal. It means that a 1.5% H2O2 solution has significantly lower bactericidal efficacy than both 2.5% NaOCl and 2% CHX. These results are consistent with that of some other studies that confirmed the significantly lower efficacy of hydrogen peroxide as an antimicrobial agent when compared to NaOCl . Ohara et al.(44) Showed that 0.3% H2O2 can kill all bacteria in 15 minutes. This diversity in results may be due to differences in the time of irrigant application in these two studies. Brown et al.(45) showed that hydrogen peroxide as a root canal irrigant can dissolve necrotic tissue and dentin debris and suggested that hydrogen peroxide be used in combination with sodium hypochlorite as an effective irrigant. Also, the combination of these two substances creates bubbles that help clear the root canal.

Totally, the results of the current study demonstrate that all of the irrigants used in this study led to a significant decrease in E. faecalis counts compared to the control group. This finding generally supports the work of some other studies in this area(45). Heling et al.(46) Investigated the antimicrobial effect of three solutions of sodium hypochlorite, chlorhexidine and hydrogen peroxide alone, in combination, and their synergistic effect. In this study, similar to ours, there was no statistically significant difference between chlorhexidine and sodium hypochlorite, and both of these substances killed large amounts of canal microorganisms. The effect of sodium hypochlorite was greater than that of hydrogen peroxide and the combination of these two substances had no greater effect than sodium hypochlorite alone but it was significantly greater than 0.3% hydrogen peroxide. Also, the effect of chlorhexidine on root surfaces was greater than that of hydrogen peroxide. However, in the deeper layers of dentin tubules, hydrogen peroxide had a greater effect. This result could be related to the permeability of the material due to its oxidation ability and molecular size.

On the other hand, the outcomes of the present study are contrary to that of Estrela et al.(16) who concluded that irrigation of infected human root canals with ozonated water, 2.5% sodium hypochlorite, 2% chlorhexidine, and the application of gaseous ozone for 20 min was not sufficient to inactivate E. faecalis. They analyzed human root canal infection from a pure culture collection. This contradictory result might be due to the differences in methodologies, bacterial invasion of root dentinal tubules, or incubation time.

Limitations

Our study was an in vitro study and should be confirmed by in vivo studies. A lack of probability sampling could have affected our results. However, it is worth emphasizing that it is extremely difficult to obtain extracted primary teeth with complete roots. We sampled bacteria from the root canals but they may harbor deep inside the dentinal tubules. We also used a pure E. faecalis suspension in our study but studies should be done focusing on polymicrobial biofilms, rather than individual microorganisms. Additional researches should be done to provide more scientific evidence, especially in primary root canals.

Conclusion

This study has shown that 2% CHX, 2.5% NaOCl and 1.5% H2O2 had statistically significant activity against Enterococcus faecalis. Since chlorhexidine has an antimicrobial effect similar to NaOCl and more than H2O2 and has a longer duration of action and less toxicity than NaOCl, it can be selected as an irrigant in the treatment of necrotic deciduous teeth. However 1.5% H2O2 has shown significantly lower bactericidal efficiency than both 2% CHX and 2.5% NaOCl, yet Several questions about this matter remain unclear. Further trials are needed to evaluate the clinical efficiency of these irrigants in necrotic primary teeth treated with pulpectomy .

Conflict of interest

The authors declare no conflict of interest with regards to the authorship and/or publication of this article.

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