



Comparison of Antibacterial Effect of Four Irrigation Solutions in Primary Root Canal Infections: A Clinical Study

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ARTICLE INFO	ABSTRACT
<p>Article Type: Original Article</p> <p>Received: 16 May 2018 Revised: 14 Sep 2018 Accepted: 27 Sep 2018 Doi: 10.22037/iej.v13i4.17900</p> <p>*Corresponding author: Nooshin Fakhari, Department of Endodontics, Dental School, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Tel: +98-913 2547889 E-mail: Nooshin_67@ymail.com</p> <p> © The Author(s). 2018 Open Access This work is licensed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International.</p>	<p>Introduction: Reducing the bacterial count from the root canal system is one of the main stages in root canal treatment. The aim of the present clinical study was to compare the antibacterial effect of four intracanal irrigants in primary endodontic infections using both microbiological culture and quantitative Real-time Polymerase Chain Reaction (qRT-PCR) technique. Methods and Materials: Forty patients with primarily infected single rooted premolars were selected and then randomly divided into 4 groups according to the intra canal irrigant used: 5.25% Sodium hypochlorite (NaOCl), Hypoclean (Ogna Laboratori Farmaceutici, Muggiò, Italy), 2% chlorhexidine gluconate (CHX) and CHX-Plus (Vista Dental Products, Racine, WI, USA). Samples were collected before and after chemomechanical preparation and were evaluated by bacterial culture and RT-PCR technique for <i>Enterococcus faecalis</i> and <i>Fusobacterium nucleatum</i>. Data analyzed by repeated measured ANOVA. The significance level was set at 0.05. Results: Four irrigation solutions significantly reduced the total numbers of cultivable bacteria ($P<0.05$). No statistically differences were found among the antibacterial effects of 5.25% NaOCl (99.93%), Hypoclean (99.94%), 2% CHX (99.77%) and CHX-Plus (99.83%) in reducing cultivable bacteria. <i>Enterococcus faecalis</i> and <i>Fusobacterium nucleatum</i> were no longer detected after preparation using four irrigants (100% reduction). Conclusions: All tested irrigants including 5.25% NaOCl, Hypoclean, 2% CHX and CHX-Plus significantly reduced the number of bacterial colonies in primary endodontic infections.</p> <p>Keywords: CHX-Plus; Endodontic Infection; <i>Enterococcus faecalis</i>; <i>Fusobacterium nucleatum</i>; Hypoclean</p>

Introduction

The presence of infection in the apical periodontitis lesions is an important criterion of endodontic treatment failure [1]. To achieve a successful endodontic treatment, bacterial eradication from the root canal system is very important. There are some reports that show the influence of the residual bacterial in the root canal after endodontic treatment procedure on the post treatment outcome [2, 3]. Therefore, choosing an effective antibacterial endodontic irrigant during root canal therapy should be the main microbiological

goal of the endodontic treatment to eradicate bacterial infection [4].

Sodium hypochlorite (NaOCl) has been used as an endodontic irrigant for many years and has paramount importance in root canal disinfection as an antimicrobial agent and an excellent tissue solvent [5-8] but its toxicity for periapical tissues is overlooked [9-11]. Also NaOCl has high surface tension that limits its penetration into dentinal tubules and irregularities of the root canal system [12]. Hypoclean is a NaOCl-based irrigant, composed of 5.25% NaOCl and two detergents (cetrimide and polypropylen glycol) [13].

Chlorhexidine gluconate (CHX) is another effective antimicrobial agent [14] that in recent decades has been used as an endodontic irrigant. This agent is suggested as an alternative to NaOCl with the same antibacterial effect and less toxicity [15, 16]. CHX-Plus is a new product from CHX which is composed of %2 CHX and a surfactant. Manufacturer states that this irrigant has not unpleasant smell and is able to kill the bacteria 2 times faster than CHX by reducing the viscosity of surface [17].

There is no clinical study on antibacterial effectiveness of Hypoclean and CHX-Plus in root canal treatment. Researchers reported that more than 500 bacterial species are present in the oral cavity, but interestingly, there are few species in the root canal that indicates the selective environment of the root canal so that some bacteria survive whereas others cannot [18]. *Enterococcus (E.) faecalis* is one of these bacteria in root canal system, that is present in primary endodontic infection and can persist in root canal system even after root canal therapy [19]. The high incidence of *E. faecalis* in persistent apical periodontitis might be related to its unique virulence factors including its ability to compete with other microorganisms, invade dentinal tubules, and resistance in nutritional deprivation. This species could survive in 500 µm depth of dentin for 3 weeks [20, 21]. The other bacterium with high prevalence in root canal infection is *Fusobacterium (F.) nucleatum* [22-24].

Bacterial culture is the most commonly employed technique for bacterial identification and pathogen detection. However, molecular techniques are more sensitive and effective than culture techniques and biochemical methods [25]. The quantitative Real-time Polymerase Chain Reaction (qRT-PCR) method provides detection and quantitative results about the exact bacterial counts in the root canal samples before and after chemomechanical root canal preparation [26, 27].

The aim of this clinical study was to compare the antibacterial effect of four irrigation solution including 5.25% NaOCl, Hypoclean, 2% CHX and CHX-Plus in teeth with primary endodontic infection using both microbiological culture and RT-PCR technique.

Materials and Methods

Patient selection

Forty patients referred to endodontic department of Tehran University of Medical Sciences for root canal treatment of single rooted premolars with one canal participated in the present study. This study protocol was approved by the ethics committee of Tehran University of Medical Sciences in Iran

(IR.TUMS.VCR.REC.1395.1232). All patients signed the informed consent form. The teeth had necrotic pulps which was confirmed with absence of response to cold test and evidence of primary endodontic infection in radiography. Patients with systemic disease and patients who had received antibiotics in past 3 months were excluded. Teeth that could not be isolated with a rubber dam or teeth with periodontal pockets deeper than 4 mm were excluded as well. All patients were treated in the postgraduate clinic of the Endodontic Department of Tehran Dental School.

All patients who agreed to participate in the study were randomly divided into 4 groups of 10 patients each. To randomize the patients, each patient was asked to choose one of 4 sealed envelopes. Based on the number of the sealed envelope, the patients received one of the irrigants. The irrigants include 5.25% NaOCl (Noradiska Dental, Tartu, Estonia), Hypoclean (OgnaLaboratoriFarmaceutici, Muggiò, Italy), %2 CHX (Cerkamed, Stalowa, Poland) and CHX-Plus (Vista Dental Products, Racine, WI, USA).

Instruments and all materials used in this study were sterile throughout the endodontic treatment and all samples were collected under strictly aseptic conditions. The teeth were isolated with a rubber dam. At first the crown and surrounding operation area were cleaned with 30% hydrogen peroxide and 5.25% NaOCl. Then 5% sodium thiosulfate was used to inactivate 5.25% NaOCl. The access cavity was prepared with high-speed diamond under manual irrigation with sterile saline solution. Procedures were performed aseptically.

For sample collection, 3 sterile paper points were used successively in full length of the canal that was determined by apex locator (I-Root Apex Locator, META, Chungcheongbuk-do, South Korea) and confirmed by using periapical radiography. Every paper point remained in the root canal for 1 min. The samples which were collected before and after irrigation were transferred into 1 mL thioglycollate broth (Quelab, Quebec, Canada) and immediately were cultured on Crystal-Violet Erythromycin (CVE) agar and brain-heart infusion (BHI, Merck, Darmstadt, Germany) in the Microbiology Department of Tehran University of Medical Sciences.

All groups were prepared in 20 min by using Mtwo files (VDW, Munchen, Germany) according to the manufacturer's instructions. Then 2 mL of each irrigant was used to rinse the canals after each instrument. At the end of preparation and before the second sampling, 5 mL 0.5% sodium thiosulfate was used in NaOCl and Hypoclean groups for 60 sec to inactivate the irrigants. CHX and CHX Plus were inactivated by Tween 80 and alpha-lecithin. The second sampling was taken from the canal as mentioned earlier. Then all teeth were obturated with gutta-

percha (Aryadent, Tehran, Iran) and AH-26 silver free sealer (Dentsply, DeTrey, Konstanz, Germany) by lateral compaction technique. At the end of procedure, the access cavities were sealed with Zonalin™ (Kemedent, UK) and the patients were referred to the Operative Dentistry Department of Tehran University of Medical Sciences for coronal restoration.

Bacterial culture procedure

The transport medium containing the samples was shaken for 1 min. Fifty microliters of each sample were plated onto BHI medium and CVE agar using sterile spreaders to culture nonselective aerobes and anaerobes; respectively. The plates were incubated at 37°C in both aerobic and anaerobic atmosphere for up to 7 days. After this period, colony-forming units (CFUs) were visually quantified for each plate. Biochemical differential diagnosis tests were used to distinguish various bacterial colonies [28].

Real Time-PCR analysis

The total DNA of the samples was isolated using YTA Genomic DNA Extraction Min Kit (Yektatajhez, Tehran, Iran) according to the manufacturer's protocol. Before DNA extraction, DNase was added to all samples to eliminate free

DNAs. The primers and probes used in Real-Time PCR were selected to detect and quantify *E. faecalis* (Genesig Standard kit, Genesig, UK) and *F. nucleatum* (FusNucdtec-qPCR kit, GPS, Alicante, Spain). Real-time polymerase chain reaction was performed based on the Step One Plus Real-Time PCR System (ABI, California, USA) with amplification conditions for *F. nucleatum* as follows: 95°C/15 sec for denaturation followed by 60°C/60 sec for 40 cycles. Amplification conditions for *E. Faecalis* was: 95°C/10 sec for denaturation followed by 60°C/60 sec for 50 cycles. Then the quantification of each bacterium was calculated.

Statistical analysis

The results were analyzed using SPSS for Windows version 17.0 (SPSS, Chicago, IL, USA). After checking the normal distribution of the results, comparison between the experimental groups was performed by applying Repeated measure ANOVA of General Linear Model. Mean of data was used for analysis. Between subject factor was experimental group (NaOCl, Hypoclean, CHX, CHX-Plus) and repeated factor was the colony count before and after using irrigation solutions. The significance level for all tests was set at 0.05.

Table 1. Mean of total bacterial load (CFUs/mL) in samples taken before and after chemomechanical preparation in 4 irrigation groups ($n=10$)

Groups	Before treatment	After treatment
NaOCl	5.5×10^5	70
Hypoclean	4.8×10^5	60
CHX	5.2×10^5	80
CHX-Plus	6.4×10^5	90

Table 2. Number of *Enterococcus faecalis* in samples of teeth with primary endodontic infection taken before and after chemomechanical preparation using 4 irrigation solutions

Groups (N)	Before treatment			After treatment		
	Min	Max	Mean	Min	Max	Mean
NaOCl (3)	0	1864174.59	9641568	0	0	0
Hypoclean (2)	0	83.19	9.1760	0	0	0
CHX (1)	0	121.56	12.15	0	0	0
CHX-Plus (3)	0	54.28	5.4280	0	0	0

Data from quantitative polymerase chain reaction analysis; *Number of samples that were positive before chemomechanical preparation

Table 3. Number of *Fusobacterium nucleatum* in samples of teeth with primary endodontic infection taken before and after chemomechanical preparation using 4 irrigation solutions

ups (N)	Before treatment			After treatment		
	Min	Max	Mean	Min	Max	Mean
NaOCl (5)	0	9943698.00	995168.74	0	0	0
Hypoclean (4)	0	1108.68	141.25	0	0	0
CHX (3)	0	1967.33	261.63	0	0	0
CHX-Plus (4)	0	9718.42	1658.65	0	0	0

Data from quantitative polymerase chain reaction analysis; *Number of samples that were positive before chemomechanical preparation

Results

Bacterial culture

Bacterial cultures showed presence of bacteria in all root canal samples before preparation. After preparation using 5.25% NaOCl, Hypoclean, 2% CHX and CHX-Plus, the cultivable bacteria decreased significantly ($P < 0.004$) (Table 1). All irrigation solutions reduced the cultivable bacteria more than 99%. There was no significant difference among 5.25% NaOCl (99.93%), Hypoclean (99.94%), 2% CHX (99.77%) and CHX-Plus (99.83%) in reducing colony number of the bacteria.

Biochemical tests identified *Streptococci*, *Micrococcus*, *Staphylococcus* and *Fusobacterium* species in root canals with primary endodontic infection before preparation. *E. faecalis* was not detected in bacterial cultures and *Fusobacterium* species were detected in 4 of 40 samples. After chemomechanical preparation using four tested irrigants only *Streptococci* species were detected in the specimens.

Real-time PCR

The Real-time PCR analysis of the root canal samples before root canal preparation using irrigation solution revealed the presence of *E. faecalis* and *F. nucleatum* in 9 and 16 specimens; respectively. After root canal preparation, *F. nucleatum* and *E. faecalis* species were no longer detected in four groups. There was no difference among four irrigants in terms of eradicating these two species. The number of *E. faecalis* and *F. nucleatum* in the positive samples before and after using irrigation solutions are shown in Tables 2 and 3.

Discussion

The present clinical study compared the antibacterial effect of 5.25% NaOCl, Hypoclean, 2% CHX and CHX-Plus during chemomechanical preparation in teeth with primary endodontic infection. Before preparation, all root canal samples were positive for the presence of many cultivable bacteria including aerobes and anaerobes. This finding supported the polymicrobial feature of primary endodontic infections reported by other investigations [24, 29].

Root canal preparation using four irrigation solutions showed an extremely significant reduction of the cultivable bacteria and *F. nucleatum* and *E. faecalis* species in all the root canal samples. These results confirmed the essential role of instrumentation by using antimicrobial irrigation in reducing the bacterial populations in infected root canals and are in agreement with the previous studies [16, 30-32].

Bacterial culture is a routine technique for bacteria detection in microbiology. However, an important concern of such methods is the fact that about one half of the endodontic bacteria could not grow and have not been detected by standard culture techniques [33]. Furthermore, culture technique is not suitable for detection of bacteria in low number and culture-difficult species like anaerobic bacteria. To overcome to these problems, molecular techniques were developed to detect uncultivated bacteria, culture-difficult species and minimum number of bacteria.

Polymerase chain reaction (PCR) assay is used for assessment of clinical samples and detection and identification of bacteria at the DNA level. An established method for precise detection and quantification of bacterial DNA is RT-PCR. This technique allows exact determination of nucleic acid levels of known microbial species by monitoring the fluorescent signals per cycle [34]. Studies have shown that the sensitivity of RT-PCR is 41 fold more than bacteria detected using bacterial culture and colony counting techniques especially for anaerobic species [25, 35].

We used DNA-based RT-PCR screening in the present study to detect *E. faecalis* and *F. nucleatum*. These bacteria are cultivable but don't survive or grow well in culture. *E. faecalis* has been commonly reported as recovered species from root canals whenever the endodontic treatment is not successful. Sundqvist *et al.* [21] found that 38% of the recovered bacteria after root canal treatment was *E. faecalis*. On the other hand *F. nucleatum* has the unique properties like the ability to survive even under difficult stresses and resistance to medications applied in the endodontic procedures [36]. Also, this species has been recovered from infected root canals with high prevalence and is one of the most common species in post-instrumentation samples [37]. So these two species selected to study the ability of irrigants for eliminating the root canal infection.

There is no clinical study that compares the antibacterial effect of Hypoclean and CHX-Plus with other endodontic irrigants. Some *in vitro* studies reported Hypoclean has more antibacterial effect compared to NaOCl [38, 39]. According to the result of this study the antibacterial effect of Hypoclean was the same as NaOCl. In the study by Mohammadi *et al.* [40], 5.25% NaOCl and Hypoclean were more effective against the microorganisms tested compared to 2% CHX. Their *in vitro* study showed depending on the taxa of bacteria, that NaOCl and Hypoclean have different abilities to eliminate the bacteria. NaOCl was the most effective irrigant against *E. faecalis* and Hypoclean was the preferred irrigant against *C. albicans*, *P. aeruginosa*, and *L. casei*.

In the present study, %2 CHX and CHX-Plus had the same antibacterial effect against *E. faecalis*. This result corresponded with the *in vitro* study by Williamson, *et al.* [41] who showed the same effectiveness of %2 CHX and CHX-Plus for eradication of *E. faecalis*.

In this study, four used irrigants showed comparable results regarding the bacterial elimination from infected root canals *in vivo*. This indicates that these agents can be assumed as equally effective antibacterial irrigants during chemomechanical preparation.

In the present study, only two culture-difficult species of bacteria detected by PCR method; further studies for detecting bacteria in low number and uncultivable bacteria of the primary infected root canal samples should be considered.

Conclusion

Four irrigation solutions including 5.25% NaOCl, Hypoclean, 2% CHX and CHX-Plus significantly reduced the intracanal bacteria. There was no difference in antibacterial effect of these irrigation solutions in single rooted premolars with primary endodontic infection.

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Conflict of Interest: 'None declared'.

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