Antibacterial Effectiveness of 2 Root Canal Irrigants in Root-filled Teeth with Infection: A Randomized Clinical Trial

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Abstract

Introduction: This study compared the antibacterial effects of 1% sodium hypochlorite (NaOCl) and 2% chlorhexidine digluconate (CHX) during retreatment of teeth with apical periodontitis. **Methods:** Root canal–treated teeth with apical periodontitis were randomly distributed into 2 groups. Bacteriological samples were taken from the canals before (S1) and after (S2) preparation using either NaOCl or CHX irrigation and after calcium hydroxide medication (S3); 16S ribosomal RNA gene-based real-time quantitative polymerase chain reaction was performed to quantify total bacteria, streptococci, and *Enterococcus faecalis*. **Results:** Forty-nine teeth were available for analysis (NaOCl, $n = 20$; CHX, $n = 29$). Bacterial DNA occurred in all S1 samples, streptococci in 57% and *E. faecalis* in 6%. The total bacterial counts decreased from S1 to S2 in both groups ($P < .01$) but were higher in S3 than S2 ($P < .01$). Thirty-five percent of the teeth in the NaOCl group were positive in S2, decreasing to 20% in S3. In the CHX group, 41% were positive in S2, decreasing to 31% in S3. The bacterial load in S1 influenced the incidence of bacteria in S2 ($P < .01$). Streptococci were significantly reduced in both groups, and *E. faecalis* was found in only 1 S2 sample and not in S3. No significant difference between NaOCl and CHX was found. **Conclusions:** NaOCl and CHX both reduced bacterial counts and the number of infected canals. Intracanal medication with calcium hydroxide reduced the number of canals with persistent infection but resulted in overall larger bacterial counts in the cases positive for bacteria. The effectiveness of antimicrobial treatment can be influenced by the initial bacterial load. *(J Endod 2016;42:1307–1313)*

Key Words

16S ribosomal RNA gene, calcium hydroxide, chlorhexidine, endodontic retreatment, post-treatment apical periodontitis, quantitative real-time polymerase chain reaction, sodium hypochlorite

Significance

This study evaluates the clinical efficacy of 2% chlorhexidine in comparison with 1% sodium hypochlorite used as irrigants in the treatment of infected root-filled teeth. We show that both irrigants are similarly efficient in bacterial reduction and removal.

Post-treatment apical periodontitis is an infectious disease in root canal–treated teeth caused mainly by persistent intraradicular infection (1). Persistent infections are more common in teeth with inadequate treatments (2), but even some adequately treated teeth fail. The main cause of failure in these cases is bacterial persistence in anatomically challenging areas, such as lateral canals, isthmi, apical ramifications, and dentinal tubules (3, 4). The lower success rate for retreatment when compared with the initial treatment of teeth with apical periodontitis (5) indicates that achieving proper root canal disinfection during retreatment may be more difficult. Because the treatment outcome is negatively influenced by the presence of bacteria at the time of root filling (6, 7), the ultimate goal during root canal treatment or retreatment is to eradicate bacterial infection.

Mechanical instrumentation needs to be accompanied by a root canal irrigant with antimicrobial properties to reduce the intracanal bacterial populations (8–10). Sodium hypochlorite (NaOCl) in concentrations ranging from 0.5%–5.25% has been widely used as a root canal irrigant. It has pronounced antimicrobial activity and the ability to dissolve organic matter (11). However, NaOCl has an adverse effect on vital tissues, and it is toxic to periradicular tissues (12). Chlorhexidine digluconate (CHX) exhibits broad-spectrum antimicrobial activity against endodontic bacteria (13) and substantivity to dentin (14, 15), but it lacks a tissue-dissolving ability (11). CHX may be less irritant to vital tissues (16). Most of the *in vitro* studies have indicated that increasing the concentration of CHX from 0.12%–2% improves the antimicrobial efficacy (17, 18). NaOCl and CHX may differ in their effects on the various members of the endodontic microbiota (13, 17, 19). This may have clinical significance because there are significant differences among the bacterial communities found in retreatment cases compared with primary endodontic infections (20).

Several *in vivo* studies have compared the antimicrobial effectiveness of CHX and NaOCl with conflicting results (21–26). Most of these studies investigated the antibacterial effects by culture-dependent methods. Culture-dependent studies have limitations related to low sensitivity and the inability to detect many difficult-to-grow or uncultivable bacteria (20). Culture-independent molecular microbiology methods can overcome these shortcomings of culture-dependent techniques (27). Few studies

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have used molecular methods to compare the antibacterial effectiveness of NaOCl and CHX (23, 25, 26). Using the reverse-capture checkerboard assay, Rôças and Siqueira (23) found no significant difference between 2.5% NaOCl and 0.12% CHX in terms of the incidence of bacterial persistence after irrigation. When comparing 2.5% NaOCl and 2% CHX in a quantitative polymerase chain reaction (qPCR), Rôças et al (26) also found no significant differences between them. Another study used qPCR to evaluate total bacteria and showed that 2.5% NaOCl was significantly more effective than 2% CHX gel (25). To our knowledge, there are no studies comparing the antibacterial effectiveness of these root canal irrigants in root canal–treated teeth with apical periodontitis using culture-independent molecular approaches such as qPCR.

The aim of this clinical study was to compare the antibacterial efficacy of 1% NaOCl and 2% CHX used as root canal irrigants in teeth with post-treatment apical periodontitis as evaluated by a molecular microbiology approach. Counts of total bacteria, *Streptococcus* species, and *Enterococcus faecalis* were evaluated before and after chemomechanical preparation and also after calcium hydroxide intracanal medication by means of qPCR.

Materials and Methods

Patient Selection

Sixty-seven consecutive patients (39 men and 28 women; mean age = 50 years; range, 21–91 years) presenting to the endodontic clinic at the School of Dentistry, University of Oslo, Oslo, Norway, and in a private practice limited to endodontics were invited to participate in this study. All treatments were performed by 1 of the authors (H.Z.). All patients exhibited post-treatment apical periodontitis either in a single-rooted tooth or in 1 root with a single canal from a multirooted tooth. Teeth with gross carious lesions, fractures involving the periodontium, and/or periodontal pockets more than 4 mm deep were excluded from the study. For all included cases, the quality of the root fillings and coronal restorations were regarded as technically adequate. Patients were not included in the study if they had diabetes, human immunodeficiency virus infection, or other immunocompromising conditions or received antibiotic therapy within the previous 3 months. On admission, cases were randomly distributed into NaOCl and CHX groups by the flipping of a coin. This randomization process resulted in 29 teeth (43%) in the NaOCl group and 38 teeth (57%) in the CHX group. Approval for the study protocol was obtained from the Regional Ethics Committee of the University of Oslo. The study and associated risks were explained to the patients, and written informed consent was obtained.

Treatment and Sampling Procedures

A rubber dam and the aseptic technique were used throughout endodontic treatment. Before rubber dam isolation, supragingival plaque was removed by scaling and cleansing with pumice. Caries and/or coronal restorations were removed with sterile high-speed and low-speed burs. After rubber dam application, the operative field, including the tooth, clamp, and surroundings, were disinfected with 3% hydrogen peroxide followed by 2.5% NaOCl. After completing the access opening with sterile burs under aseptic conditions, the operative field, including the pulp chamber, was cleaned and disinfected once again. NaOCl was neutralized with 5% sodium thiosulfate (Sigma-Aldrich, St Louis, MO), and sterility control samples (SR1) were taken from the tooth surface with a sterile Omni Swab (Whatman FTA, Sigma-Aldrich) with an ejectable head. The swab was transferred to a cryotube containing Tris-EDTA buffer (10 mmol/L Tris-HCL, 1 mmol/L EDTA, pH = 7.6) (Sigma-Aldrich) and immediately placed in a Labtop cooler (−20°C Naglene Labtop cooler, Sigma-Aldrich) or directly to a freezer (−80°C). Samples in the Labtop cooler were later transferred to a freezer. For the inclusion of a tooth in the study, sterility control samples had to be uniformly negative after polymerase chain reaction with universal primers 8f and 1492r (28, 29).

The coronal two thirds of the root filling was mechanically removed with Gates-Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland), PreRaCe burs (FKG Dentaire, La Chaux/de/Fonds, Switzerland), and/or hand files. The canal was filled with sterile saline solution with care to not overflow; a sterile #15 K-file (Dentsply Maillefer) was placed to a level approximately 1 mm short of the apical foramen, based on diagnostic radiographs and with the aid of the Root ZX electronic apex locator (J Morita Corp, Tokyo, Japan); and a gentle filing motion was applied. A larger endodontic file was used to engage the root canal filling material. On withdrawal from the canal, the instrument was cut with a presterilized wire cutter, and the fragment with attached root filling material was put in a cryotube. In addition, the root canal walls were filed with sterile saline without suction, and the entire canal content was absorbed onto 3 sterile paper points and transferred to Tris-EDTA buffer (S1). Each paper point was inserted to the full length of the instrumented canal and left for about 1 minute. Apical preparation was completed to the working length with hand nickel-titanium files (NitiFlex, Dentsply Maillefer) in a back-and-forth alternating rotation motion. Master apical files ranged from #40 to #60 depending on both the root anatomy and the initial diameter of the root canal. The irrigants used were 1% NaOCl solution in 29 cases and 2% CHX solution in 38 cases. In each group, the total volume of irrigant was 10 mL delivered by a 30-G needle (Max-i-Probe, Dentsply Maillefer). Each canal was dried using sterile paper points, and then 5 mL 5% sodium thiosulfate or a mixture of 0.07% lecithin, 0.5% Tween 80, and 5% sodium thiosulfate solutions (Sigma-Aldrich) was used to neutralize any residual NaOCl or CHX, respectively (23, 26, 30). Subsequently, the root canal walls were filed, and a postinstrumentation sample (S2) was taken from the canal using sterile paper points as described earlier. Calcium hydroxide paste mixed with sterile saline was placed with engine-driven Lentulo spiral fillers (Dentsply Maillefer) in the entire root canal extent and packed with paper points. A 2-mm plug of Cavit-G (3M ESPE, St Paul, MN) was placed in the coronal portion of the canal orifice. On top of that, a thick layer of IRM (Denstply, York, PA) was used as a temporary filling. The dressing was left in place for an average of 25 days (median = 18 days).

At the second visit, the tooth was isolated with a rubber dam, and disinfection of the operative field was performed as mentioned earlier. The temporary restoration was removed, and the operative field, including the pulp chamber, was cleaned and disinfected once again. Sterility control samples were taken (SR2). The intracanal dressing was removed with sterile saline and with gentle filing using an endodontic instrument under magnification in a microscope. The canal was dried with sterile paper points, and the canal walls were gently filed with a Hedstrøm instrument. Sterile saline was placed in the canal, and a postmedication sample was taken using 3 sterile paper points (S3). The root canal was then irrigated with 10 mL either 1% NaOCl or 2% CHX, dried, and obturated with gutta-percha and AH Plus (Denstply) sealer using the cold lateral compaction technique. The tooth was sealed with Cavit and IRM, and a final radiograph was taken.

DNA Extraction and qPCR Analysis

DNA from clinical samples was extracted by using the MasterPure DNA isolation kit from Epicenter (MCD85201; Epicenter Illumina, Cambridge, UK). To quantify the levels of total bacteria, *Streptococcus* species and *E. faecalis* before and after treatment procedures, 16S ribosomal RNA gene target qPCR was performed with Power SYBR Green PCR Master MIX (Applied Biosystems, Foster City, CA) on an ABI 7500