Disinfecting Effects of Rotary Instrumentation with Either 2.5% Sodium Hypochlorite or 2% Chlorhexidine as the Main Irrigant: A Randomized Clinical Study

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Abstract

Introduction: This randomized clinical study compared the antibacterial effects of irrigation with either 2.5% sodium hypochlorite (NaOCl) or 2% chlorhexidine (CHX) during the preparation of infected root canals with rotary nickel-titanium instruments. **Methods:** The root canals of 50 single-rooted teeth with apical periodontitis were prepared by using BioRaCe rotary instruments (FKG Dentaire, La Chaux-de-Fonds, Switzerland) and irrigation with either 2.5% NaOCl (n = 25) or 2% CHX (n = 25). Samples were taken from the canal at baseline (S1) and after (S2) chemomechanical preparation. DNA was extracted from the clinical samples, and the reduction of the levels of total bacteria and streptococci was evaluated by means of a 16S ribosomal RNA gene-based quantitative polymerase chain reaction assay. **Results:** All S1 samples were positive for the presence of bacteria. After chemomechanical preparation using either 2.5% NaOCl or 2% CHX, 44% and 40% of the root canals still had detectable bacteria, respectively. As for total bacterial counts, a mean number of 3.7×10^5 bacterial cell equivalents was present in S1 samples from the NaOCl group, with a substantial reduction in S2 to a mean of 5.49×10^2 cell equivalents ($P < .001$). In the CHX group, a mean bacterial load of 8.77×10^4 cell equivalents occurred in S1, with a significant reduction in S2 to a mean of 2.81×10^3 cells ($P < .001$). The differences in both the presence/absence and quantitative data were not statistically significant ($P > .05$). Both irrigation protocols were highly effective in reducing the levels of *Streptococcus* species ($P < .001$). **Conclusions:** No significant difference was observed for the clinical antibacterial effectiveness of rotary preparation using either 2.5% NaOCl or 2% CHX as the main irrigant. *(J Endod 2016;42:943–947)*

Key Words

Apical periodontitis, chlorhexidine, endodontic treatment, root canal irrigation, sodium hypochlorite

Proper control of the root canal infection is crucial for optimal treatment outcome (1, 2). Sterilization of the root canal system is virtually impossible to achieve with current instruments, substances, and techniques; thus, the major realistic microbiological goal of endodontic treatment is to reduce intracanal bacterial populations to levels that are compatible with periradicular tissue healing (3). Chemomechanical procedures play an essential role in reducing the bacterial burden in the main root canal, which is the area of the system that harbors the largest amounts of bacteria (4). The mechanical effects induced by instruments and the streaming of irrigants remove large amounts of bacteria, but the use of an antimicrobial irrigant solution is required to optimize disinfection (1, 5).

For many years, sodium hypochlorite (NaOCl) has been the most commonly used endodontic irrigant. Its great acceptance among clinicians is related to its strong antibacterial and tissue-dissolving abilities (6, 7). However, NaOCl has some disadvantages, including tissue toxicity and development of serious complications when apically extruded (8, 9). This has prompted the search for alternative irrigants, such as chlorhexidine (CHX). CHX does not have tissue-dissolving ability but has good antibacterial activity against oral bacteria (7, 10) and possesses the property of substantivity (11), which may result in prolonged antibacterial effects. Many *in vitro/ex vivo* studies have compared the antibacterial activities of NaOCl and CHX, some reporting that NaOCl is superior (7, 12, 13), some that CHX is superior (10, 14), and some that they are similar (15–18). As for clinical studies, comparative data are scarce and also inconclusive, as clearly shown by a recent systematic review (19). Of the studies using culture as the method of microbiologic analysis, 2 reported that NaOCl is superior (20, 21), and the other 2 showed no significant differences between them (22, 23). Of the molecular studies comparing the clinical antibacterial effects of the 2 substances, 1 showed no significant differences (24), whereas the other showed that NaOCl was superior (20). All these previous clinical studies used hand instrumentation, and so far no study has compared the antibacterial effectiveness of NaOCl and CHX as the irrigants using contemporary rotary nickel-titanium instruments.

This clinical study was performed to compare the antibacterial effectiveness of 2.5% NaOCl and 2% CHX used as irrigants during the chemomechanical preparation of infected root canals associated with primary apical periodontitis lesions. The canals were

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prepared with rotary nickel-titanium instruments, and the reduction of the levels of total bacteria and streptococci was evaluated by means of a quantitative culture-independent molecular microbiology assay.

Materials and Methods

Subjects

The study population consisted of subjects attending the endodontic clinic at the School of Dentistry, Estácio de Sá University, Rio de Janeiro, RJ, Brazil, for evaluation and treatment of apical periodontitis from March 2009 to December 2010. Only teeth with a single root and a single canal and presenting with carious lesions, necrotic pulps confirmed by pulp tests, and clinical and radiographic evidence of asymptomatic apical periodontitis were included in the study. Reasons for exclusion included extensive destruction of the tooth crown by caries, root or crown fracture, previous endodontic treatment, symptomatic teeth, presence of deep periodontal pockets (>4 mm), and patients who received antibiotic therapy within the previous 3 months. Approval for the study protocol was obtained from the Ethics Committee of the Estácio de Sá University, and informed consent was obtained from all individuals or their parents.

Sample Size Calculation and Randomization

A reduction in bacterial counts using either NaOCl or CHX as the irrigant was the primary outcome to be compared in this study. Therefore, sample size calculation was based on this parameter. By using STATISTICA v8.0 software (StatSoft, Tulsa, OK), sample size calculation revealed that a minimum of 21 teeth per group would be sufficient to show a 5% difference in bacterial counts with a power of 90%. Therefore, teeth were randomly distributed into 2 groups of 25 each according to the type of irrigant used, 2.5% NaOCl or 2% CHX. Randomization with equal proportion allocation was obtained by drawing lots. It was not feasible to blind patient and treatment provider because of the recognizable odor of NaOCl.

Treatment and Sample Taking Procedures

Root canal samples were taken under strict asepsis. Caries and defective coronal restorations were removed with sterile high-speed and low-speed burs. Next, the tooth crown was cleansed with pumice, and a rubber dam was placed. The tooth and surrounding field were disinfected by a protocol using 3% hydrogen peroxide followed by 2.5% NaOCl solution. After completing the access with another sterile bur under sterile saline irrigation, the operative field, including the pulp chamber, was once again disinfected. NaOCl on the tooth surfaces was inactivated with sterile 5% sodium thiosulfate. Sterility control samples were taken from the cavosurface angle of the access cavity with sterile paper points before sampling from the root canal. For inclusion in the study, all teeth should have presented negative sterility control samples in a polymerase chain reaction (PCR) assay with universal 16S ribosomal RNA (rRNA) gene-based primers. Three patients were excluded from the experiment and replaced by another 3 individuals according to the inclusion/exclusion criteria and randomization process.

The initial sample (S1) was taken from the root canal as follows. Sterile saline solution was placed in the canal with care to not overflow, and a sterile size 15 hand K-file was introduced to a level approximately 1 mm short of the root apex, as determined by a diagnostic radiograph. A gentle filing motion was applied, and then sterile paper points were consecutively placed in the canal to the same level and used to soak up the fluid in the canal. Each paper point was left in the canal for at least 1 minute, and the total number of points used was the amount sufficient to dry the canal. Paper points were transferred aseptically to cryotubes containing Tris-EDTA buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH = 7.6) and immediately frozen at –20°C.

Chemomechanical procedures were completed at the same appointment. After irrigation of the canal with 1 mL 2.5% NaOCl or 2% CHX, the working length was established 1 mm short of the apical foramen by using an electronic apex locator (Novapex; Forum Technologies, Rishon Le-Zion, Israel) and confirmed with radiographs. The canal was initially enlarged and the apical patency established by using a size 20 hand K-type file. Preparation was performed using BioRaCe instruments (FKG Dentaire, La Chaux-de-Fonds, Switzerland). The master apical files used for apical preparation ranged from BR5 (40/.04) to BR7 (60/.02). Patency of the apical foramen with small hand files was checked throughout the procedures. Irrigation with either NaOCl or CHX was conducted throughout the intracanal procedures using disposable syringes and NaviTip needles (Ultradent, South Jordan, UT) inserted up to 3 mm short of the working length. A total volume of 15 mL of either irrigant was used per canal.

After preparation, the root canal was dried and then flushed with 5 mL of either a 10% sodium thiosulfate solution or a mixture of 0.07% lecithin, 0.5% Tween 80 (Sigma Aldrich, São Paulo, SP, Brazil), and 5% sodium thiosulfate to neutralize any residual NaOCl or CHX, respectively. Postinstrumentation (S2) samples were taken from the root canals as described previously.

The smear layer was removed, and the canal was dried with paper points and medicated with a calcium hydroxide paste. One week later, the root canal was filled with gutta-percha and sealer by the lateral compaction technique.

Quantitative Real-time PCR Analysis

Samples were thawed to the room temperature, and DNA was extracted by using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's directions. The levels of total bacteria and *Streptococcus* species were quantified before and after chemomechanical procedures by using a 16S rRNA gene-based quantitative real-time PCR (qPCR) assay with Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) on an ABI 7500 Real-time PCR instrument (Applied Biosystems). The total reaction volume was 20 μL, and the primers, cycling conditions, standard curve construction, controls, and data analysis were performed as described previously (25). Sensitivity of the qPCR assay was set at 10^2 bacterial cell equivalents. Data are presented as the counts of cell equivalents per sample. All measurements were taken in triplicate for samples, standards, and controls.

Statistical Analysis

Quantitative data from qPCR for total bacteria and streptococci were analyzed as follows. Intragroup bacterial reduction was evaluated by comparing S1 and S2 samples with the Wilcoxon matched pairs test. S1 samples were compared between groups by using the nonparametric Mann-Whitney *U* test, which showed no statistically significant difference ($P > .05$). Therefore, data on the absolute counts in S2 were used for comparisons between groups using the Mann-Whitney *U* test. The presence/absence data in S2 from the 2 groups were compared using the Fisher exact test. The significance level was set at $P < .05$ for all tests.

Results

Of the 50 individuals included in the study, 27 were male and 23 female, with a mean age of 29 years (range, 13–52 years). All of them reported no significant systemic condition. Each individual contributed 1 tooth.

Bacteria were detected in all samples taken before treatment as revealed by qPCR with universal 16S rRNA gene-based primers. After