Interaction between Octenidine-based Solution and Sodium Hypochlorite: A Mass Spectroscopy, Proton Nuclear Magnetic Resonance, and Scanning Electron Microscopy–based Observational Study

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Abstract

Introduction: Octenisept (OCT; Schülke & Mayr, Nordersdedt, Germany), an antimicrobial, antibiofilm agent and a promising root canal irrigant, can be potentially combined with sodium hypochlorite (NaOCl) during endodontic treatment. The aim of this study was first to identify the precipitate formed on the interaction between OCT and NaOCl and secondly to compare its effect on dentinal tubules with that of precipitate formed on combining chlorhexidine (CHX) and NaOCl. **Methods:** This observational study was conducted in 3 stages. Initially, the color changes and precipitate formation were assessed when the test solution 0.1% OCT and 5.2% NaOCl were mixed. Color changes were compared with those observed when 2% CHX was mixed with 5.2% NaOCl. The residue obtained on combining OCT and NaOCl was subjected to proton nuclear magnetic resonance (^{1}H NMR) and mass spectrometric (MS) analysis. In the final stage, dentinal surfaces irrigated alternatively with OCT and NaOCl were compared using scanning electron microscopy (SEM) with the dentinal surface irrigated with CHX and NaOCl. **Results:** The OCT-NaOCl mixture changed in color from initial milky white to transparent over time, whereas the CHX-NaOCl mixture showed an immediate peach-brown discoloration. ^{1}H NMR and MS analysis established that the whitish precipitate obtained on combining OCT and NaOCl solutions correlated with the structure of phenoxyethanol (PE). SEM revealed dense precipitate occluding the dentinal tubules with the CHX and NaOCl group, whereas the precipitate was sparse and partially occluded in the OCT and NaOCl group. **Conclusions:** The whitish precipitate formed with the OCT-NaOCl mixture was identified as PE, a compound already present in OCT, and it partly occluded the dentinal tubules. *(J Endod 2017;43:135–140)*

Key Words

Chlorhexidine, irrigants, nuclear magnetic resonance, Octenidine, precipitate, sodium hypochlorite

Significance

Octenidine-based solution (OCT) is a broad-spectrum antimicrobial agent with antibiofilm properties. The combined irrigation of OCT with sodium hypochlorite (NaOCl) results in the precipitation of phenoxyethanol, which is an antimicrobial agent. Hence, there is potential for combined irrigation of OCT and NaOCl.

A combination of techniques involving mechanical preparation, irrigation with antimicrobial agents, and intratubular disinfection with medication is used to achieve disinfection of the root canal system. Sodium hypochlorite (NaOCl) is widely used as a root canal irrigant because of its tissue-dissolution potential and antibacterial action (1). However, dentin, tissue debris, blood, and their combination can rapidly limit its antibacterial potential (2). Some studies have attempted to circumvent this by combining NaOCl with other irrigants such as chlorhexidine gluconate (CHX) (2).

CHX, a bisbiguanide with sustained antibacterial properties, has been tested as an irrigant along with NaOCl (3). This sequence was found to result in an orange-brown precipitate, the toxicity of which is widely debated (3, 4). This precipitate can adhere tenaciously to the root canal wall, with possible implications for the integrity of obturation (5). Contrary to popular belief, CHX is also more cytotoxic than NaOCl (6). Hence, there is still a need for a different supplemental irrigant to act synergistically with NaOCl.

Octenisept (OCT; Schülke & Mayr, Nordersdedt, Germany) is an antiseptic containing 0.1% octenidine hydrochloride and 2% phenoxyethanol (PE). Octenidine is a positively charged bis-pyridinamine with a broad spectrum of antibacterial, antifungal,

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2016.09.015

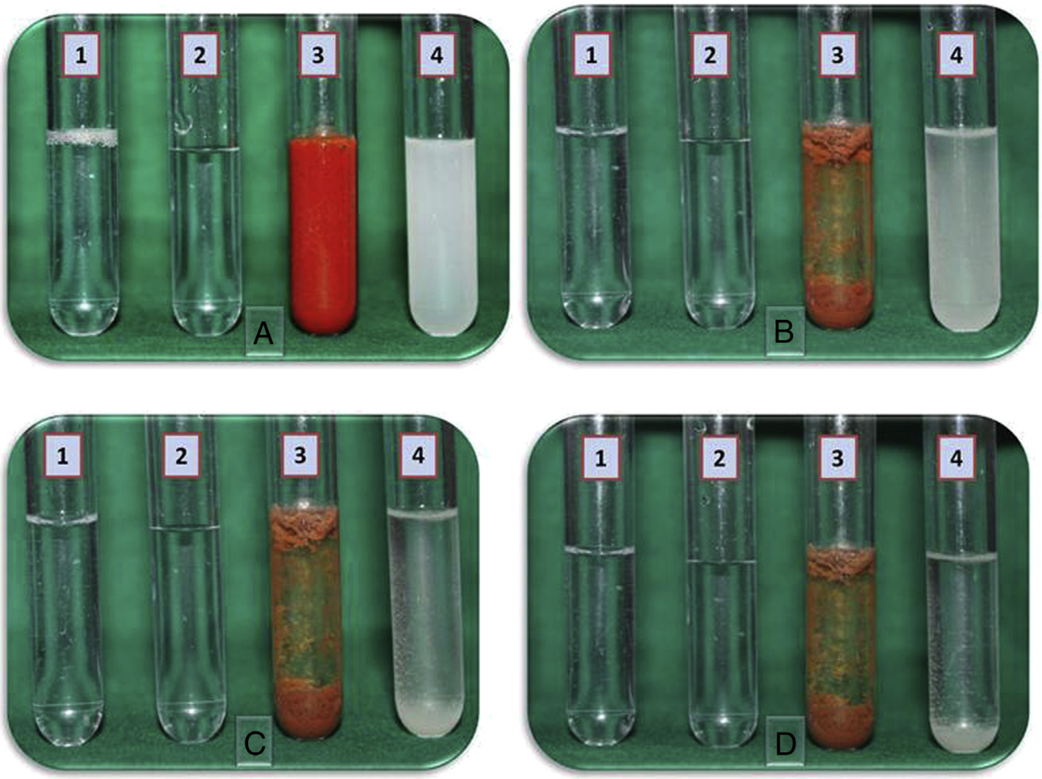

Figure 1. Color change and precipitate formation in the tested solutions. The peach-brown color in the mixture of CHX and NaOCl (tube 3) intensified over time with precipitation (*A*: 0 hours, *B*: 12 hours, *C*: 24 hours, and *D*: 48 hours). The color change of the OCT and NaOCl mixture (tube 4) changed from milky white immediately at (*A*) 0 hours to clear transparent solution by the (*D*) 48th hour with the settling of the whitish precipitate. The negative control tube 1 (OCT) and tube 2 (CHX) remained transparent for the entire period.

and some antiviral properties (7). It is particularly capable of inhibiting the formation of biofilm and disrupting fully formed biofilm even in the presence or absence of serum protein (8). It has been shown to produce a dramatic reduction of intraoral biofilm and endodontic pathogens within a short time (9, 10). However, its potential as a stand-alone irrigant is limited because of its poor tissue-dissolving properties (11). Initial observations have shown that mixing OCT and NaOCl results in an immediate whitish precipitate, the origin and nature of which is unknown. Hence, the aim of this study was first to identify the compound in the whitish precipitate using mass spectrometric (MS) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy and secondly to assess its effect on dentinal tubules qualitatively compared with the precipitate formed with CHX and NaOCl using scanning electron microscopy (SEM).

Materials and Methods

For the evaluation of color changes and precipitate formation, commercially available solutions of 2% CHX (Asep RC; Staedman Pharmaceuticals, Chennai, India), Octenisept, and 5.25% NaOCl (Associated Clinical Aids Pvt Ltd, Calicut, India) were used in the study. A sterile polystyrene round-bottom tube (tube 1) (Falcon; Thermo Fischer Scientific, Waltham, MA) was filled with 2 mL OCT alone and tube 2 with 2 mL 2% CHX. One milliliter each of 2% CHX and 5.25% NaOCl was placed in tube 3. Tube 4 received 1 mL OCT and 1 mL 5.25% NaOCl. All 4 tubes were kept at 36.5°C with 95% humidity for 1 week. These tubes were observed by an independent observer for precipitates or color changes every 15 minutes for the first 2 hours and after that at 12-hour intervals for 1 week. The process was repeated 3 times for verification.

Scanning Electron Microscopic Analysis

For additional confirmation and to simulate a clinical setting, root canals in 6 lower premolar teeth extracted for periodontal or orthodontic reasons were accessed and prepared by a crown-down technique using the ProTaper Universal rotary file system (Dentsply Maillefer, Ballaigues, Switzerland) up to size F3. The working length was determined using size 10 K-files (Mani Inc, Tochigi, Japan). Instrumentation with rotary files was kept 1 mm short of the exit from the apical foramen. The root canals were irrigated with 5 mL 5.25% NaOCl solution after each instrumentation and change of file. The 17% EDTA solution was flooded in the canals for 2 minutes for smear layer removal followed by a final flush with distilled water to remove the traces of EDTA.

After drying with paper points, 3 of the teeth (group 1) were subjected to irrigation with 10 mL 5.25% NaOCl followed by 10 mL 2% CHX. The remaining 3 teeth (group 2) were irrigated with 10 mL 5.25% NaOCl and 10 mL OCT. The root surfaces were grooved with a disk and split using a chisel; the dentinal surface was observed for the presence of precipitates by SEM (Model S2400; Hitachi, Tokyo, Japan).

Spectroscopic (^{1}H NMR, MS) Analysis

To identify the precipitate, a mixture of 10 mL OCT and 10 mL 5.25% NaOCl solution was transferred into in a 50-mL glass beaker and allowed to stand for 7 days. The solution was then twice extracted with 5 mL each of ethyl acetate to remove any organic species present from the mixture. The extracts were combined, washed with saturated brine to remove most of the adhered water present, and dried by adding anhydrous sodium sulphate (1 mg). A thin-layer chromatography of the dried extract showed only a single spot, indicating the presence of only a