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Alexidine versus chlorhexidine for endodontic irrigation with sodium hypochlorite

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ABSTRACT

Objective: The objective of this study was to chemically evaluate precipitate formation on irrigation by different concentrations of chlorhexidine (CHX) and alexidine (ALX) with sodium hypochlorite (NaOCl). **Materials and Methods:** Six test tubes were prepared with 1 ml of 4% NaOCl. One milliliter of 2%, 1%, 0.5%, and 0.25% ALX was added to the first four, and in the last two, 1 ml of 2% CHX and 0.2% CHX was added, respectively. Samples were observed for color changes or precipitates at multiple time intervals. All solutions were then centrifuged at 1000 rpm for 10 min and re-examined for precipitates. This process was repeated twice. Fifty freshly extracted premolars were biomechanically prepared, dried, divided into two groups, and irrigated with 10 ml of 4% NaOCl and 10 ml of 2% ALX (Group 1) and 10 ml of 4% NaOCl and 10 ml of 2% CHX (Group 2). These samples were sectioned and observed for precipitates on the dentinal surfaces by scanning electron microscopy (SEM). **Results:** The color of the solution of ALX and NaOCl stayed transparent and no precipitate was observed. A color change was noted immediately on mixing CHX and NaOCl which did not change with time. Precipitates were only observed in the solutions of CHX with NaOCl and after centrifuging them. SEM views also showed dense precipitates covering the dentinal surface and occluding the dentinal tubules in Group 2. **Conclusion:** The interaction of ALX and NaOCl does not produce precipitates which together with its better antimicrobial action make ALX a more effective and safer replacement for CHX as an adjunctive endodontic irrigant.

Key words: Alexidine, chlorhexidine, para chloro aniline, precipitate, scanning electron microscope

INTRODUCTION

Microorganisms remaining in or recolonizing the root canal after treatment are the main causes of endodontic failure.[1] A combination of thorough cleaning and shaping eradicates these and their by-products from the root canal.[2] Cleaning is accomplished with various antimicrobial irrigants, used alone or as admixtures, to enhance the effect of mechanical instrumentation.[3]

An ideal irrigant should have a broad-spectrum antimicrobial activity, dissolve necrotic pulp, inactivate endotoxins, serve as lubricant, prevent smear layer formation, and be nontoxic and nonirritating to the oral tissues.[3] When used in combination, they should unleash their full potential on targets in the root canal rather than on each other. The most common

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endodontic irrigants are sodium hypochlorite (NaOCl) and chlorhexidine (CHX).

NaOCl has a broad-spectrum antimicrobial activity and dissolving action on necrotic tissues.[4] Its disinfecting efficiency depends on the concentration of dissociated hypochlorous acid which exerts its germicidal effect by an oxidative action on sulfhydryl groups of bacterial enzymes, killing the bacterial cells.[5] However, it lacks prolonged antimicrobial activity once bound to a surface (substantivity, a property essential for effective root canal disinfection), and microorganisms such as *Enterococcus faecalis* are resistant to it.[6-8]

CHX, a potent bisbiguanide, at concentrations of 0.1%–0.2% is bacteriostatic (used as an antiplaque agent)[9] and at higher concentrations of 2% is bactericidal (used as a root canal irrigant).[10] Since it lacks tissue-dissolving properties, it is only a supplemental irrigant.[11] Through its affinity to dental hard tissues[12] and antimicrobial substantivity,[11-14] it is the most effective final irrigant.

2.5% NaOCl with 0.2% CHX together has been advocated to have better antimicrobial effect than each component alone[3,15] and use of NaOCl irrigation throughout instrumentation and CHX as final irrigant is commonplace. A concern about this is the formation of an orange-brown precipitate in the canals[16] which not only stains the dentin but also hampers the seal of obturation, leading to leakage of precipitates into the periapex.[17]

Alexidine (ALX), a bisbiguanide-like CHX, is a disinfectant which has recently gained much attention for its longer substantivity and potency against *E. faecalis*. This is through its greater affinity for the major virulence factors such as bacterial lipopolysaccharide (LPS) and lipoteichoic acid (LTA),[18] making it a potentially ideal final irrigating solution.

This study was thus aimed to compare the safer final irrigant in the chemical interaction of NaOCl with different concentrations of ALX and CHX by assessing color changes and precipitate formation.

MATERIALS AND METHODS

In this *in vitro* study, 6 test tubes were prepared with 1 ml of 4% NaOCl (kept refrigerated to maintain the chloride ion activity, confirmed immediately before the experiment by chlorine test paper). In the first four, 1 ml of 2%, 1%, 0.5%, and 0.25% ALX was added, and in the last two, 1 ml of 2% CHX and 0.2% CHX was added, respectively. All solutions were stirred with a glass rod and kept at 36.5°C in 95% humidity for 2 weeks. Samples were observed for color changes or precipitate formation in the reaction solutions immediately, after 15 min, 30 min, 1 h, 2 h, and at 1 week. All 6 solutions were then centrifuged at 1000 rpm for 10 min and observed for precipitates. This entire process was repeated twice for confirmation.

To reconfirm our results, 50 single-rooted human mandibular premolar teeth that were devoid of developmental defects and had been extracted due to periodontal or orthodontic reasons were obtained. Extracted carious teeth, those that had undergone restorative or endodontic treatment, fractured teeth, and teeth with attrition, abrasion, or erosion were excluded from the study. The working length was determined for these specimens using #10-K file and prepared to 40/.06 size using X2 ProTaper Next nickel–titanium rotary instruments using crown-down technique. Irrigation was performed using 5 mL of 4% NaOCl solution with 25-mm, 30-gauge needles after each instrumentation. The root canal surfaces were conditioned for smear layer removal by 17% ethylenediaminetetraacetic acid (EDTA) for 2 min, followed by a final flush with distilled water to remove any trace of the demineralizing solution. All teeth were dried and divided into two groups of 25 each. Group 1 was irrigated alternatively with 10 ml of 4% NaOCl and 10 ml of 2% ALX and Group 2 with 10 ml of 4% NaOCl and 10 ml of 2% CHX for 3 min. The prepared teeth samples were split with a chisel and observed for precipitates on the dentinal surfaces by scanning electron microscopy (SEM). The central beam was directed to the surface area of each tooth under ×1000 magnification.

The results were subjected for appropriate statistical analysis using the Statistical Package for the Social Sciences Software (SPSS version 16, IBM, Chicago, IL, USA). Categorical data were analyzed by Kruskal–Wallis test and Chi-square test for any difference between the groups. Fisher's exact test was used to compare the outcome between the two groups. $P \leq 0.05$ was considered statistically significant.

RESULTS

There was no color change when NaOCl was mixed with ALX, while there was an immediate change in color (dark peach brown and light peach brown) on mixing NaOCl with 2% and 0.2% CHX, respectively, which did not change with time [Figure 1a]. At the end of a week, NaOCl mixed with different concentrations of ALX centrifuged at 1000 rpm for 10 min showed no precipitate formation but showed precipitate formation with CHX [Figure 1b], which was statistically significant ($P < 0.001$). The same results were obtained each time the test was repeated.

SEM analysis showed the absence of precipitates [Figure 2a] in Group 1 (NaOCl irrigated with ALX) while Group 2 (NaOCl irrigated with CHX) showed dense precipitates covering the dentinal surface and occluding the dentinal tubules [Figure 2b] in all 25 specimens, which was statistically significant.

DISCUSSION

Biomechanical cleaning and shaping of the root canals reduces the bacteria but does not completely eliminate them[19] making canal irrigants important. Combinations of irrigants used together or sequentially give better antimicrobial effect, but sometimes, their interaction could be detrimental to the outcome of the root canal therapy.[3,15]

Although new as an irrigant, ALX has found many uses in medicine and is successfully used as a mouth rinse agent in periodontics due to its better residual salivary antibacterial activity than traditional CHX.[20] Recently, ALX dihydrochloride has been identified as the first bisbiguanide compound with anticancer properties and thus its potential use of as an apoptosis-promoting anticancer agent.[21]

ALX and CHX both suppress bacterial membrane-induced cell activation at concentrations lower than that used for topical applications. These cationic irrigants disrupt the integrity of bacterial cytoplasmic membrane and thereby result in the leakage of the intracellular contents. They not only bind to LPS but also to LTA from Gram-positive bacteria. In fact, ALX has higher affinity than CHX for both these compounds which has been determined in the past by fluorescence displacement assay and isothermal calorimetric titration.[18]

2% ALX has an antibacterial effect similar to 2% CHX against *E. faecalis*, suggesting that both irrigants can be used for a supplementary final rinse before intracanal medication or as canal soaking agents before canal obturation in infected root canals.[22] Furthermore, 2% and 1% ALX used for 1 min has been proved to provide longer antimicrobial substantivity against *E. faecalis* than 2% and 0.5% CHX[22,23] although higher concentrations of ALX (>2%) could cause moderate cytotoxicity against human gingival fibroblasts and should be avoided.[22]

The interaction of irrigants showed no color change with NaOCl and ALX, and immediate color change when CHX and NaOCl were mixed is in agreement with previous studies.[24,25] No precipitate was observed on reaction of different ALX concentrations with NaOCl in test tubes. Even when 2% ALX was irrigated in the root canal with 4% NaOCl and observed under SEM, no precipitate formed.[24] However, in the CHX and NaOCl group, a peach brown precipitate gathered at the bottom of the test tube in both 2% and 0.2% CHX groups after centrifugation, and on irrigation in root canals, the precipitate occluded the dentinal tubules when observed under SEM analysis. Our findings were consistent with existing literature that the thickness and quantity of precipitate formed by the interaction of CHX and NaOCl is directly proportional to the concentration of NaOCl.[26] It has been reported that CHX when placed in an aqueous solution slowly hydrolyses into smaller fragments and forms parachloroaniline (PCA)[27] through a substitution of the guanidine group in the CHX molecule.[28]

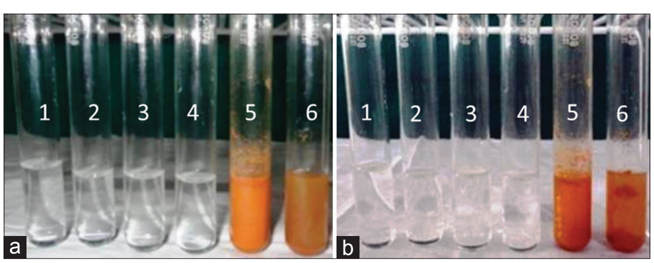

Figure 1: (a) Color changes only observed in Test Tube 5 (2% chlorhexidine) and 6 (0.2% chlorhexidine) (b) precipitate formation observed only in Test Tube 5 (2% chlorhexidine) and 6 (0.2% chlorhexidine)

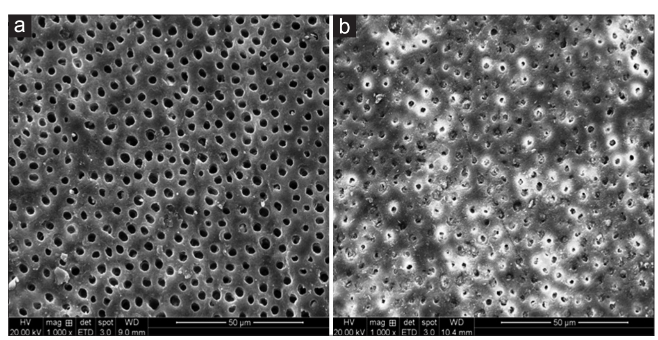

Figure 2: Scanning electron microscopy images (a) no precipitate in alexidine samples – Group 1 (b) precipitate formation clogging the dentinal tubules in the chlorhexidine samples – Group 2

Structurally, ALX has two hydrophobic ethylhexyl groups, whereas CHX contains p-chlorophenyl end groups. Consequently, ALX cannot produce a PCA precipitate when mixed with NaOCl.[29]

Precipitate formation can also be explained by the acid–base reaction of NaOCl and CHX. CHX, a dicationic acid (pH: 5.5–6.0), donates protons whereas NaOCl is alkaline and accepts them. This exchange results in an insoluble neutral substance, the "precipitate." This mixture of NaOCl/CHX displayed the same breakdown pattern as CHX alone, suggesting that CHX was still present in the precipitate.[26] This acts as a chemical smear layer and compromises the dentin permeability by occluding them in the coronal and middle thirds of the canal.[25] It affects the diffusion of intracanal medication. An additional interface between the sealer and the dentin also affects the obturation seal, especially with resin sealers in which a hybrid layer is required.[16,17,30] Removing NaOCl by aspiration and paper points showed no significant reduction in precipitate formation as dentin and its tubules harbor enough residual NaOCl to react with the CHX, raising potential concerns.[25]

Earlier studies have demonstrated the formation of precipitate by gas chromatography–mass spectrometry (MS), X-ray photon spectroscopy (XPS), and time-of-flight secondary ion MS (TOF-SIMS). TOF-SIMS was used to determine the presence of PCA, but the amount was determined with the help of XPS.[24] The chemical composition of the precipitate has been confirmed in the past by Beilstein and HCl tests.[17] It is known that PCA and its degradation products are toxic and carcinogenic.[31,32] As an aromatic amine, short-term exposure to PCA results in cyanosis, which is a manifestation of methemoglobin formation. Severe methemoglobinemia has been reported in neonates exposed to PCA produced as a breakdown product of CHX resulting from incubator heat.[33]

Many authors used an intermediate irrigant after NaOCl and before CHX such as saline, 50% citric acid, and 14% EDTA, to prevent the formation of PCA, but none of those prevented it.[34] Citric acid used as the intermediate irrigant had the least amount of PCA formation in the root canals. Absolute alcohol as an intermediate rinse between NaOCl and CHX has been employed to prevent the formation of the precipitate as it is volatile, tensioactive, and highly electronegative and can penetrate deep to remove the residual NaOCl from the canals.[17] ALX precludes any such intermediary irrigant use and saves important chair time.

The clinical relevance of the observed color change and its associated tooth staining can be explained by the viscid peach brown precipitate formed by mixing NaOCl and CHX. It stains dentin, as observed, and acts as a residual film which cannot be completely removed from the root canal. In contrast, the association of ALX and NaOCl does not produce any precipitate. ALX also possesses better antimicrobial action. ALX thus clearly surpasses CHX as a root canal irrigant, sequentially or in combination with NaOCl, and can be used as its replacement.

CONCLUSION

The interaction of ALX and NaOCl does not produce precipitates and with its outmatched antibacterial properties is a more effective and safer replacement for CHX as an adjunctive endodontic irrigant.

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Conflicts of interest
There are no conflicts of interest.

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