Influence of Irrigation with NaOCl and Chlorhexidine on Microleakage

SUMMARY

Background: Irrigation during endodontic therapy is required in order to remove debris, tissue remnants, microbes and smear layer. Sodium hypochlorite (NaOCl) and Chlorhexidine (CHX) are the most commonly used irrigants. Although they are reported to have good antimicrobial effects, both have limitations. Hence, a combination of NaOCl and CHX has been proposed to compensate for these limitations. However, this association forms a dense, orange-brown precipitate that stains walls of the pulp chamber. The aim of this study was to clarify in vitro if this precipitate affects the microleakage of endodontic sealers.

Material and Methods: Extracted human teeth were used for this study. The teeth were cut at the height of the cervix and instrumented with NiTi rotary instruments. They were then divided into 2 experimental groups. In the first group (Group A) irrigations were performed with 2ml NaOCl 1%, 1ml EDTA 17% and 1ml CHX 0.2% and in the second (Group B) with 2ml NaOCl and 2ml CHX. Following this, they were obturated with gutta-percha and roth sealer. The microleakage was determined using a fluid filtration method. The measurements were repeated a month later. All analyses were performed using Fisher exact test.

Results: Microleakage of Group A was lower than microleakage of Group B but the difference was not statistically significant.

Conclusion: The precipitate that is formed by NaOCl and CHX did not affect microleakage of endodontic sealers.

Keywords: Irrigation; Precipitate; Microleakage; Sodium hypochlorite; Chlorhexidine

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Introduction

The primary aim of root canal treatment is to eliminate bacteria from the infected root canal and to prevent re-infection[1,2]. Although a mechanical action of instruments can reduce the number of bacteria, complete canal disinfection is difficult because of the complexity of the internal root canal anatomy[2,3]. In addition, mechanical instrumentation forms a smear layer on the canal surface[3]. As a result, irrigation is required to remove debris, tissue remnants, microbes and the smear layer[3]. A variety of irrigants have been used in an attempt to achieve these aims but no single preparation has been found to be completely predictable or effective[1].

Sodium hypochlorite (NaOCl) is the irrigant most commonly used during endodontic therapy in a concentration ranging from 0.1% to 5.25%. It is efficient in dissolving organic tissues as well as eliminating microorganisms[2-6]. Its germicidal ability is related to the formation of hypochlorous acid when in contact with organic debris[2]. However, in high concentrations, NaOCl is toxic and can cause inflammation in the periapical tissues, whereas in low concentrations it is ineffective against specific microorganisms[6,7]. Moreover, NaOCl is unable to completely remove the smear layer, it does not impart antimicrobial substantiveness, it corrodes surgical instruments and has a very unpleasant odor[2,6,7].

An alternative irrigant is chlorhexidine gluconate (CHX). CHX is a cationic bisbiguanide with a broad-

spectrum antimicrobial action that acts by absorbing onto microbial cell walls or by disrupting them, causing leakage of intracellular components[1,8]. It has an antibacterial efficacy comparable to that of NaOCl[9,10], while also being effective against certain NaOCl resistant bacterial strains[9,11]. In addition, CHX has the advantages of having both substantiveness[13] and low level of toxicity[12]; however, the inability of CHX to dissolve organic matter is a perceived drawback[14].

Both NaOCl and CHX have limitations despite their reported good antimicrobial effects[8]. Hence, a combination of NaOCl and CHX has been recommended to enhance their properties, on the basis that the antimicrobial effect of 2.5% NaOCl and 0.2% CHX used in combination is better than that of either solution alone[10]. However, this association forms a dense, orange-brown precipitate that stains the walls of the pulp chamber[2,7,15,16]. This insoluble precipitate is difficult to remove from the canal, occludes the dentinal tubules[3] thus preventing penetration of the intra-canal medicaments and compromises the seal of the obturated root canal[6]. Additionally, its presence imparts colour to the canal wall and causes tooth discoloration affecting aesthetic appearance[6,17]. Moreover, it has been reported that when mixed with NaOCl, CHX hydrolyzes and forms para-chloroaniline (PCA), a toxic and carcinogenic product[18].

In order to eliminate formation of the precipitate, the use of intermediate flushes of saline or distilled water in greater volumes to enhance dilution effect on NaOCl has been suggested, or to eliminate the formation of precipitate by flushing away the remaining NaOCl with absolute alcohol before using CHX as the final irrigant; However, in this case, the biocompatibility of alcohol with the periapical tissues remains a concern[2]. Irrigation with EDTA and/or drying with paper points in order to remove the NaOCl has also, been proposed[7,8,16,19]. Kuruvilla[10] managed to dissolve the precipitate with methanol. Alternatively, 0.1 mol/ml acetic acid also manages to dissolve the precipitate[10].

The aim of this study was to clarify *in vitro* if the precipitate caused by the NaOCl and CHX combination affects microleakage of endodontic sealers.

Material and Methods

40 single-rooted extracted human teeth were used for this study. Each tooth was placed in NaOCl for 2 hours for surface disinfection, and then stored in distilled water until use. 35 teeth of these were cut at the height of the cervix with a high speed bur leaving only the root. The working length was determined with a #10 K-File by introducing into the canal until the tip of the file was visible at the apical foramen and then subtracting 1mm. All the resulting 35 samples were subsequently prepared

by rotary instrumentation with Protaper instruments using a F3 File as the finishing file. During instrumentation, irrigation was performed using NaOCl between each instrument. Then, these 35 teeth were randomly divided into 2 experimental groups of 15 teeth (A and B) and one positive control group of 5 teeth (C). The remaining 5 teeth from the initial 40 composed the negative control group (D).

The samples of group A were irrigated with 2ml NaOCl 2.5%, 1ml EDTA 17% and 1ml CHX 0.2 %. After each irrigation, root canals were dried with paper points. The teeth of group B were irrigated with 2ml NaOCl and 2ml CHX. Finally, the teeth of groups A and B were obturated using one point technique with gutta-percha Protaper points NoF3 and Roth sealer (Roth International LTD). Positive controls were left unfilled.

Thus the following groups were created:

Group A: This group comprised 15 teeth, which were prepared, irrigated with NaOCl, EDTA and CHX and obturated with gutta-percha

Group B: This group comprised 15 teeth, which were prepared, irrigated with NaOCl and CHX, and obturated with gutta-percha.

Group C: This group comprised 5 teeth which were prepared, irrigated with NaOCl but left unfilled.

Group D: This group comprised the first 5 teeth, which were not instrumented; these constituted the negative control group.

Microleakage was evaluated by using a modified fluid filtration study design previously reported by Pashley and Depew (1986). The specimens were attached to a plastic tube. Cyanoacrylate cement was applied circumferentially between root and plastic tube to fix the specimens tightly. A 20 HL glass micropipette was connected to the plastic tube on the outlet side of the specimen. Distilled water was used to fill all pipettes, syringes and plastic tubes. The whole set-up was then placed in a water bath (20°C) and, using a syringe, the air bubble was adjusted to a suitable position within the capillary. A pressure of 30 kPa (0.296 atm) was applied on the coronal side. A 5-minute pressurization preload of the system was completed before taking readings. The position of the bubble was recorded. The quality of the seal of each specimen was measured at 2, 4, 6 minutes. The amount of microleakage was recorded in the units of HL/cm H_2O per minute. The measurements were repeated a month later.

The data was evaluated using SPSS software (Statistical Package for the Social Sciences). Analysis of Fisher's exact test was used to perform the comparison. The significance level was set at 5%.

Results

The results are presented in table 1 (immediate microleakage) and table 2 (microleakage 30 days later).

The positive controls showed fluid movement throughout the length of the canals (Group C), while the root canals in the negative control group did not display any fluid leakage (Group D). The microleakage of Group A after 30 das was lower than microleakage of Group B but the difference was not statistically significant. A month later, despite an expected rise in microleakage in both groups, the results were the same: there was no statistical difference between the groups.

Table 1. Immediate microleakage of group A and B

time	2 min		4 min		6 min	
	Group A	Group B	Group A	Group B	Group A	Group B
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	CL*	0	CL*	0	CL*
4	0	0	0	0	0	0
5	CL*	CL*	CL*	CL*	CL*	CL*
6	0	CL*	0	CL*	0	CL*
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	CL*	0	CL*	0	CL*
10	0	CL*	0	CL*	0	CL*
11	0	0	0	0	0	0
12	CL*	0	CL*	0	CL*	0
13	CL*	CL*	CL*	CL*	CL*	CL*
14	0	CL*	0	CL*	0	CL*
15	0	0	0	0	0	0

* - CL: cross leakage

Table 2. Microleakage for group A and B after 30 days

Time	2 min		4min		6 min	
	Group A	Group B	Group A	Group B	Group A	Group B
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	CL*	0	CL*	0	CL*
4	0	0	0	0	0	0
5	CL*	CL*	CL*	CL*	CL*	CL*
6	0	CL*	0	CL*	0	
7	0	0	0	0	0	0
8	CL*	CL*	CL*	CL*	CL*	CL*
9	CL*	CL*	CL*	CL*	CL*	CL*
10	0	CL*	0		0	
11	0	0	0	0	0	0
12	CL*	0	CL*	0	CL*	0
13	CL*	CL*	CL*	CL*	CL*	CL*
14	0	CL*	0	CL*	0	CL*
15	0	CL*	0	CL*	0	CL*

* - CL: cross leakage

Discussion

It is well established that biomechanical cleaning and shaping of the root canal system using files and antibacterial irrigants reduces the bacteria load but no irrigant can completely eliminate all organic and inorganic matter and at the same time impart a substantive residual antibacterial property to the canal wall dentin. A combination of NaOCl and CHX has been proposed in order to enhance their antimicrobial properties and to improve the chemical cleaning of root canal[10]. Although such a combination of irrigants may enhance their antimicrobial properties chemical interactions among the irrigants have to be considered.

As already mentioned, the combination of NaOCl and CHX results in the formation of a precipitate. Basrani et al[7] determined that this formation is immediate and independent of the concentration of NaOCl and CHX. The lowest concentration of NaOCl to cause the formation of precipitate was 0.19%[7]. When the NaOCl came in contact with CHX precipitate was formed and a colour change occurred immediately, which did not change with time[7]. As concentration of NaOCl increased, the colour varied from peach to brown and the precipitate thickened[7].

The precipitate covers the dentin walls and affects the patency of the dentinal tubules[3,17]. Bui et al[3] found a statistically significant reduction in the number of patent dentinal tubules in the experimental groups when compared with the negative control group, but the obstruction of dentinal tubules was not found to be significant in the apical third. There were no significant differences among all the experimental and control groups. This might be due to the fact that the apical third is more difficult to irrigate[3]. However, Akisue et al[8] assert

that the combination of 1% NaOCl and 2% CHX solutions results in formation of a flocculate precipitate that acts as a chemical smear layer reducing the permeability of the dentine in the apical third.

Formation of the precipitate could be explained by the acid-base reaction occurring when NaOCl and CHX are mixed[7]. CHX, a di-cationic acid (pH 5.5-6.0) has the ability to donate protons. NaOCl is alkaline and can accept protons from the di-cationic CHX[7]. This proton exchange results in the formation of a neutral insoluble substance, referred to as the "precipitate".

In regard to actual composition of the precipitate, there is no general agreement and more research needs to be done to clarify this[17]. Basrani et al[7] used x-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectrometry (TOF-SIMS) to identify this precipitate. They found that the precipitate contains a significant amount of para-chloroaniline (PCA). It has been reported that, when placed in an aqueous solution, CHX slowly hydrolyzes and forms para-chloroaniline. This occurs through the substitution of the guanidine group in the CHX molecule. Basrani's findings indicated that when mixed with NaOCl, the CHX molecules become hydrolyzed into smaller fragments, each forming a by-product. It has been suggested that the first bonds to be broken in this reaction are between carbon and nitrogen because of the low bond dissociation energy existing between the 2 atoms. Molecules with low bond disassociation energies are more prone to breakdown. This disassociation results in formation of PCA, among other fragments. PCA can further degrade to 1-chloro-4-nitrobenzene[3]. Krishnamurthy et al[2] maintain that PCA is the main product of the interaction of NaOCl and CHX, with the molecular formula NaC_6H_4Cl as analyzed by mass spectrometry. The presence of PCA was confirmed in this study by the Beilstein test for the presence of chlorine and the HCl solubility test for the presence of aniline[2]. The presence of chlorine in the para position of the benzene ring was finally confirmed using the nuclear magnetic resonance imaging technique[2]. However, Thomas and Sem[15] claimed that the reaction mixture of NaOCl and CHX does not produce PCA in any measurable quantity, and further investigation is needed to determine the chemical composition of the brown precipitate[15]. In addition, Nowicki and Sem[21] using one dimensional and dual dimensional NMR found that the precipitate consists of at least 2 sub-products of CHX, smaller than CHX, neither of them being PCA.

PCA has industrial uses in pesticides and dye sand has been demonstrated to be carcinogenic in animals[22]. Its degradation product, 1-chloro-4-nitrobenzene, is also a carcinogen[3]. As an aromatic amine, the primary toxic effect is methemogloblin formation[18]. Short-term exposure of humans to PCA results in cyanosis, which is a manifestation of methemoglobin formation[18]. Toxicological studies in rats and mice have shown that the haemopoietic system is

the major target for PCA[18,22]. Chabra et al[18] conducted a 90-day study and found that methaemoglobin formation and accompanying haemolytic anaemia, extra-medullary haematopoiesis, and splenomegaly were indicative of erythrocyte toxicity and regenerative anaemia. They reported PCA to be carcinogenic in rats due to increased sarcomas in the spleen[18]. In male mice, there was an increase in hepato-cellular carcinomas and haemangiosarcomas of the spleen[18]. The amount of precipitate left behind is unclear. Concern exists that this precipitate might be absorbed onto the root surface and can slowly leach into the periapical tissues[3].

In addition to these concerns, the presence of this precipitate on the root surface might affect the seal of an obturated root canal, especially with resin sealers in which a hybrid layer is required[3]. Vivacqua-Gomez et al[6] studied the coronal microleakage after root-canal treatment using different endodontic irrigants and found that the combination of NaOCl with CHX showed the worst results when compared to 1% NaOCl and 17% EDTA, 2% CHX gel, distilled water[6]. Our findings are not in accordance with this study as we found that the irrigation method did not significantly affect the microleakage.

However, the precipitate formed is also of clinical relevance because of staining, reduction in the action of antiseptic solutions and possible leaching of PCA into the periapical tissue. In order to eliminate all these drawbacks, while still taking advantage of the combined action of NaOCl and CHX, various strategies have been employed. Hence, the use of intermediate flushes of saline or distilled water in greater volumes to enhance the dilution effect on NaOCl or to prevent its formation by flushing away the remaining NaOCl with absolute alcohol before using CHX as the final irrigant has been suggested. However, in this case, biocompatibility of alcohol with the periapical tissues remains a concern[2]. Irrigation with EDTA and/or drying with paper points in order to remove the NaOCl have also been proposed[7,8,16,19]. Kuruvilla and Kamath[10] managed to dissolve the precipitate with Methanol. In addition, the use of 0.1mol/ml acetic acid, which also dissolves the precipitate, has been proposed[10].

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