The effect of using an alternative irrigant between sodium hypochlorite and chlorhexidine to prevent the formation of para-chloroaniline within the root canal system

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Abstract

Aim To determine if the formation of para-chloroaniline (PCA) can be avoided by using an alternative irrigant following sodium hypochlorite but before chlorhexidine.

Methodology Fifty-five single-rooted teeth were decoronated, instrumented to size 40, .06 taper whilst being irrigated with 14% ethylene-diamine-tetra-acetic acid (EDTA) and 6% NaOCl. Samples were then randomly divided into three experimental and two control groups. Group 1 was irrigated with saline followed by 2% chlorhexidine gluconate (CHX). Group 2 was irrigated with 50% citric acid (CA) followed by 2% CHX. Group 3 was irrigated with 14% EDTA followed by 2% CHX. The chemical identity and quantification of the PCA in the formed precipitate was determined using gas chromatography/mass spectrometry (GC/MS).

Results All experimental groups contained PCA. The mean level of PCA for group 1 (sterile saline) was 229 ng mL⁻¹, group 2 (citric acid) 72 ng mL⁻¹ and group 3 (EDTA) 400 ng mL⁻¹, respectively. A significant difference was found between the saline and EDTA groups and the negative control (P < 0.05). Although no statistical significance was found between the negative control and citric acid group, PCA was still present in this experimental group.

Conclusions Citric acid used as the intermittent irrigant had the least amount of PCA formation in the canal system. Until the threshold required to cause biological damage in humans is determined, the combination of NaOCl and CHX in root canal treatment should be avoided.

Keywords: chlorhexidine, irrigation, para-chloroaniline, sodium hypochlorite.

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Introduction
Microorganisms are known to be the aetiologic agents of pulpal inflammation and apical periodontitis (Kekhashi et al. 1965, Lin et al. 2006). The purpose of root canal treatment is to eliminate bacteria from the infected canal system and to prevent recontamination. Mechanical instrumentation alone is inadequate in its ability to remove bacteria and tissue from this complex system (Bystrom & Sundqvist 1981). Thus, various irrigant solutions have been proposed to eliminate microorganisms, debris, tissue remnants and smear layer from root canal systems and dentinal tubules. It is, therefore, important to understand not only the
effects of these irrigants during treatment procedures, but also the interactions they may have with each other whilst in contact with dental and nondental human tissues.

The most common irrigant used in root canal treatment is sodium hypochlorite (NaOCl) (Zehnder 2006). It has been used in a concentration range from 0.5% to 6% (Ohara et al. 1993). Alternative irrigants such as 2% chlorhexidine gluconate (CHX) have been recommended as a less toxic alternative (Ohara et al. 1993, Yesilsoy et al. 1995). However, owing to its main limitation on tissue dissolution (Okino et al. 2004), CHX should not replace NaOCl as the irrigant of choice, but serve as an adjunctive antimicrobial agent in the final step of the irrigation process (Kuruvilla & Kamath 1998, Grawehr et al. 2004).

Recently, another concern was raised in regard to the reaction between CHX and other irrigants (Basrani et al. 2007, 2009, Rasimick et al. 2008). Specifically, the combination of NaOCl and CHX produces a brown precipitate, and the presence of ethylene-diamine-tetraacetic acid (EDTA) and CHX produces a pink precipitate forming a salt (Basrani et al. 2007, Rasimick et al. 2008). The brown precipitate forms regardless of the concentration and ratio of NaOCl and CHX mixed (Basrani et al. 2007, Marchesan et al. 2007).

Previous studies have demonstrated that the precipitate formed by NaOCl and CHX contains para-chloroaniline (PCA) (Basrani et al. 2007, 2009, 2010). Clinically, this precipitate may occlude the dentinal tubules (Bui et al. 2008, Akisue et al. 2010), impairing cleaning and sealing of the root canal system. From a biological standpoint, it may have negative effects on human tissues with the major concern being the potential to be carcinogenic as demonstrated in animal studies (Van der Bijl et al. 1984, Chhabra et al. 1991). However, it is not known what concentration is required to cause damage in human tissues and whether this precipitate leaks out of the root canal system. Diazotization, x-ray photon spectroscopy (XPS) and time-of-flight secondary-ion mass spectrometry (TOF-SIMS) have been used to characterize the NaOCl/CHX precipitate with the conclusion that the substance formed is PCA (Basrani et al. 2007, 2009, 2010). Thomas & Sem (2010) used 1H nuclear magnetic resonance (NMR) spectroscopy to determine the reaction mixture of NaOCl and CHX. They concluded that PCA was not produced at any measurable quantity and that further investigation is needed to determine the chemical composition of the brown precipitate. Recently, Krishnamurthy & Sudhakaran (2010) tested intermediate flushes of absolute alcohol, saline and distilled water. The chemical composition of the precipitate was confirmed by Beilstein and HCl tests, and the NMR imaging technique confirmed chlorine in the para position of the benzene ring. Absolute alcohol showed no evidence of precipitate.

Based on the data from previous studies, it has been recommended to exercise caution and use an alternative irrigant between NaOCl and CHX to prevent the formation of this precipitate (Basrani et al. 2007, Bui et al. 2008). Hence, the purpose of this study was to evaluate three commonly used endodontic solutions and their effects on preventing the formation of PCA in the root canal system using mass spectrometry (MS).

Materials and methods

Fifty-five single-canal extracted human teeth were used. All teeth were decoronated and their length standardized to 15 mm. Patency was gained using a size 10 Flexofile (Maillefeer Caulk Co, Milford, DE, USA) introduced into the canal until the tip of the file was visible at the apical foramen. The working length was set at 14 mm, and a glide path was established by using size 15 and 20 Flexofilés.

The root end of each sample was then sealed with wax to create a closed system resembling the clinical situation and fluid dynamics during root canal irrigation (Marchesan et al. 2007). A tooth vise (PanaVise, Sparks, NV, USA) was used to mount and hold the tooth in the same position during instrumentation and irrigation. Immediately after mounting, the coronal access was isolated with a rubber dam and a surgical suction tip placed next to the orifice. Canal enlargement was performed with ProFile rotary files (Tulsa Dental, Tulsa, OK, USA) to size 40, .06 taper in a crown-down manner. Positive pressure irrigation was set 1 mm short of the working length. Canals were irrigated with 1.5 mL of 6% NaOCl between instruments using a 30-G side-vented needle (Max-i-Probe; Dentsply Tulsa Dental, York, PA, USA). A size 15 Flexofile was intermittently used to maintain working length. After instrumentation was completed, all teeth were irrigated with a cycle of 5 mL of 14.65% EDTA and 5 mL of 6% NaOCl. All chemical solutions were prepared by the pharmacy at the University of Washington Medical Centre based on the concentration requested. Teeth were then randomly divided into three experimental groups of 15 roots each and two controls of five roots each. Tooth type was identified and recorded accordingly.
Group 1. Sterile saline ($n = 15$)
Root canals in this group were irrigated with 5 mL of sterile saline followed by 5 mL of 2% CHX. The canals were then dried with needle aspiration and paper points.

Group 2. Citric acid ($n = 15$)
Root canals in this group were irrigated with 5 mL of 50% citric acid followed by 5 mL of 2% CHX. The canals were then dried with needle aspiration and paper points.

Group 3. EDTA ($n = 15$)
Root canals in this group were irrigated with 5 mL of 14.65% EDTA followed by 5 mL of 2% CHX. The canals were then dried with needle aspiration and paper points.

Positive control ($n = 5$)
Root canals in this group were irrigated with 5 mL of 2% CHX. The canals were then dried with needle aspiration and paper point.

Negative control ($n = 5$)
Root canals in this group were irrigated with 5 mL of 6% NaOCl. The canals were then dried with needle aspiration and paper points.

Mass spectrometry
After irrigation was completed, the apical seal was removed and teeth prepared for analysis by MS. All samples were individually soaked in 1 mL dichloromethane in glass vials to extract any PCA in the root canal system. Samples were soaked for at least 24 h in a refrigerator to prevent evaporation and allow the solvent to penetrate the tooth. A 1 µL aliquot was injected splitless into a gas chromatograph coupled with a mass spectrometer operating in selected ion monitoring mode (Agilent Technologies 6890 GC with 5973 MS: column DB-5MS, 25 m x 0.20 mm ID, 0.33 µm film thickness). The oven temperature started at 40 °C, and after a 2-min hold, it ramped to 320 °C with 20 °C min$^{-1}$ slope. The PCA eluted at retention time 9 min and ions of $m/z$ 127, 129, 92 and 65 were monitored for the presence of PCA. An external calibration was used to correlate the peak area to the PCA amount remaining in the tooth after the procedure.

Gas chromatography-MS confirms PCA presence by three part evidence, the chromatographic retention time, molecular ion with correct isotope pattern for chlorine element and an electron impact spectrum with correct fragmentation pattern and ratios. All these are identical to the standard PCA injected on the system during the method development for analysis of the study samples. The spectrum is also matching a spectrum from the NIST database (NIST/EPA/NIH 2008).

Statistical analysis
Kruskal–Wallis one-way analysis of variance on ranks was used at a 0.05 level of significance. Pairwise multiple comparison procedure was then carried out using the Dunn’s Method ($P < 0.05$).

Results
Para-chloroaniline was present in the canals of all experimental groups. Positive controls had 1626 ng mL$^{-1}$ of PCA on average whilst no PCA was detected in the negative controls. The mean level of PCA for group 1 (sterile saline) was 229 ng mL$^{-1}$, group 2 (citric acid) 72 ng mL$^{-1}$ (citric acid) and group 3 (EDTA) 400 ng mL$^{-1}$, respectively (Fig. 1). The overall mean for all experimental groups combined was 234 ng mL$^{-1}$. When comparing all the groups with the Dunn’s Method, there was no statistical significance found between the negative group and citric acid group. A significant difference was found between the saline and EDTA groups and the negative control group.
control ($P < 0.05$). Although no statistical significance was found between the negative control and citric acid group, it is important to indicate that PCA was still present in this experimental group.

To assess if root canal size and morphology had an impact on the results, the results were analysed based on tooth type. No statistically significant differences in the amount of PCA extracted from the root canal were found between the tooth types (Fig. 2).

**Discussion**

The selection of the solutions used in this investigation was based on several factors including previous studies (Krishnamurthy & Sudhakaran 2010) and the fact that these irrigants are routinely used in clinical endodontics. In addition, EDTA and citric acid are known for causing release of chlorine gas from NaOCl which may play a role in the chemical interactions (Baumgartner & Ibay 1987). Sodium thiosulfate is well known for its chemical inactivation of sodium hypochlorite. However, pilot studies demonstrated the formation of a gross precipitate and therefore it was not included in this study.

Mass spectrometry is a sensitive and versatile technique used to determine the identity and composition of molecules, and it is also widely used to quantify compounds (Hübschmann 2001). This technique ionizes chemical compounds to generate charged molecules or molecule fragments allowing measuring the mass-to-charge ratios. Whilst the sample is destroyed during analysis, a sensitive and specific detection of the components is obtained. For the purpose of this study, the nondestructive techniques as NMR spectroscopy reported in a previous study (Thomas & Sem 2010) would not have sufficient sensitivity. To minimize the limitations of an *in vitro* model, human extracted teeth were used on this study to resemble the interactions of the irrigating solutions with human dentin. The fact that EDTA had statistically more PCA present than saline and citric acid could be owing to the fact that the combination of both NaOCl/CHX precipitate and EDTA/CHX precipitate may have made it more difficult to readily flush out the PCA from the canal and occluded dentinal tubules (Bui *et al.* 2008, Akisue *et al.* 2010). It can be hypothesized that positive results obtained with citric acid could be owing to the partial release of the chlorine gas from the decomposition of NaOCl before the addition of CHX. However, this did not completely prevent the formation of PCA.

Root canal anatomy may play a role in the amount of PCA left behind in the canal system. No significant difference was detected between tooth types in the amount of PCA remaining in the canals. However, the canals were standardized by investigating only single-canal teeth, and all teeth were decoronated to achieve the same working length. Greater differences between tooth types may have been revealed had teeth with more complex anatomy including isthmuses and fins been studied. This complex anatomy provides a rational explanation regarding the failing concept of drying the canals with paper points in between irrigant solutions to prevent the formation of the precipitate.

**Conclusion**

Within the limitations of this *in vitro* study, none of the tested solutions used for intermittent irrigation prevented the formation of PCA. Citric acid used as the intermediate irrigant had the least amount of PCA formation in the canal system. Even though the amount of remaining PCA was not significantly different between the citric acid and the negative control, the combination of NaOCl and CHX in endodontic therapy should be avoided. However, further research is warranted to determine the mechanism of diffusion of PCA into periradicular human tissues and the threshold required to cause biological damage.

**Reference**


