

Does Para-chloroaniline Really Form after Mixing Sodium Hypochlorite and Chlorhexidine?

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Abstract

Introduction: Mixing sodium hypochlorite (NaOCl) with chlorhexidine (CHX) forms a brown-colored precipitate. Previous studies are not in agreement whether this precipitate contains para-chloroaniline (PCA). Tests used for analysis may demonstrate different outcomes. Purpose of this study was to determine whether PCA is formed through the reaction of mixing NaOCl and CHX by using high performance liquid chromatography, proton nuclear magnetic resonance spectroscopy, gas chromatography, thin layer chromatography, infrared spectroscopy, and gas chromatography/mass spectrometry. **Methods:** To obtain a brown precipitate, 4.99% NaOCl was mixed with 2.0% CHX. This brown precipitate was analyzed and compared with signals obtained from commercially available 4.99% NaOCl, 2% solutions, and 98% PCA in powder form. **Results:** Chromatographic and spectroscopic analyses showed that brown precipitate does not contain free PCA. **Conclusions:** This study will be a cutoff proof for the argument on PCA formation from reaction of CHX and NaOCl. (*J Endod* 2016;42:455–459)

Key Words

Brown precipitate, chlorhexidine, interaction, irrigants, nuclear magnetic resonance, para-chloroaniline, sodium hypochlorite

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Residual infected pulpal tissue, infected dentin, and bacteria remnants in the root canal can lead to failure of root canal treatment (1); therefore, irrigation along with mechanical cleaning plays an important role for success (2). Sodium hypochlorite (NaOCl) is one of the most frequently used root canal irrigation solutions with antimicrobial properties and organic tissue dissolving capacities (3–5). However, NaOCl in high concentrations is toxic and may irritate periapical tissues (6, 7). Chlorhexidine digluconate (CHX) has been introduced as a broad-spectrum antimicrobial agent for root canal disinfection as an alternative to NaOCl (8), but CHX has no tissue dissolution capacity (9). To enhance efficacy of irrigation, different irrigation solutions are used in combination with NaOCl because of their positive synergic effects on antimicrobial properties (10–12). CHX may be used as final irrigant because of its residual antibacterial effect on root canal walls (12). However, it has been suggested that NaOCl and CHX should not be mixed within the root canal, and NaOCl should be removed from the canal system before CHX is introduced. If NaOCl is present in the canal when CHX is introduced, a brown-colored precipitate is formed, which is difficult to remove from the root canal system (13) and may cause discoloration of the dental structures. This brown substance may not only block lateral canals but may also affect the seal of root canal filling (12). Some of the previously published studies claimed that the brown precipitate formed after interaction between NaOCl and CHX contains para-chloroaniline (PCA) (14–17); however, others suggested that this precipitate did not contain PCA (18). Studies that investigated this brown precipitate are shown in Table 1.

On the basis of data from previous studies (Table 1), it is unclear whether PCA is formed after interaction between NaOCl and CHX. Because PCA is known to be toxic (24) and carcinogenic (25) and may lead to methemoglobinemia (26–28), it is important to know whether PCA is formed after interaction between NaOCl and CHX. Therefore, the purpose of this study was to evaluate whether PCA is formed through the reaction of mixing NaOCl and CHX by using high performance liquid chromatography (HPLC), proton nuclear magnetic resonance (1H-NMR) spectroscopy, gas chromatography (GC), thin layer chromatography (TLC), infrared (IR) spectroscopy, and gas chromatography/mass spectrometry (GC/MS) tests.

Materials and Methods

To prepare 2.0% CHX, 10 mL fresh 20% CHX (Chlorhexidine Digluconate Solution, Lot #BCBL2494 V; Sigma-Aldrich, St Louis, MO) was diluted with 90 mL distilled water. Commercially available 4.99% fresh NaOCl solution (Hypochlorite Solution, Lot #HMBD1456 V; Sigma-Aldrich) and 98% PCA (4-Chloroaniline, Lot #BCBJ1580 V; Sigma-Aldrich) in powder form were used. Analytical grade ethyl acetate, acetonitrile (ACN), and dichloromethane were used for extraction and/or solvation. Experiments were conducted at 25°C room temperature. Ten mL 2.0% CHX was added to 10 mL NaOCl in a 50-mL glass beaker and stirred (Stirring Rod; Acrol Scientific Laboratory Systems, İstanbul, Turkey) continuously for a minute. A brown-colored precipitate was formed immediately. The brown-colored mixture was then extracted 3 times in 15 mL ethyl acetate to transfer the brown precipitate into organic solvent. Organic layer

TABLE 1. Studies about PCA Categorized by Purpose of Study, Study Design, and Outcomes

Study no.	Reference	Purpose of study		Analysis method	Study design	Outcome
		Identification of the precipitate	Removal/prevention of the precipitate			
1	Basrani et al, 2007 (14)	+	-	X-ray photon spectroscopy	PCA or isomers found in precipitate.	PCA or isomers found in precipitate.
2	Marchesan et al, 2007 (19)	+	+	Time-of-flight secondary ion mass spectrometry	Presence of Ca, Fe, and Mg in precipitate.	Presence of Ca, Fe, and Mg in precipitate.
3	Bui et al, 2008 (13)	-	+	Atomic absorption spectrophotometry	Canal walls covered by precipitate.	Canal walls covered by precipitate.
4	Basrani et al, 2009 (20)	+	-	Environmental SEM	Diazotization technique	Presence of aromatic amine in precipitate and in CHX at 45°C.
5	Basrani et al, 2010 (15)	+	-	GC/MS	PCA was found in precipitate.	PCA was found in precipitate.
6	Thomas and Sem, 2010 (18)	+	-	1H-NMR spectroscopy	No PCA was found in precipitate.	No PCA was found in precipitate.
7	Krishnamurthy and Sudhakaran, 2010 (21)	+	+	Stereomicroscopy, nuclear magnetic resonance, the Beilstein and HCl solubility tests	Precipitate contains chlorine in the para position of the benzene ring. Precipitate can be prevented by using absolute alcohol. Intermediate flushing with saline and distilled water recommended.	Precipitate contains chlorine in the para position of the benzene ring. Precipitate can be prevented by using absolute alcohol. Intermediate flushing with saline and distilled water recommended.
8	Ballal et al, 2011 (22)	-	+	HPLC	No precipitate formation when maleic acid was combined with CHX.	No precipitate formation when maleic acid was combined with CHX.
9	Mortenson et al, 2012 (17)	-	+	GC/MS	PCA was found. Citric acid is recommended as intermittent irrigant.	PCA was found. Citric acid is recommended as intermittent irrigant.
10	Kolosowski et al, 2014 (16)	+	-	Time-of-flight secondary ion mass spectrometry	PCA was found in dentin tubules.	PCA was found in dentin tubules.
11	Arslan et al, 2015 (23)	+	-	1H-NMR	PCA was found in NaOCl and CHX mixture. PCA was not found in NaOCl and Qmix mixture.	PCA was found in NaOCl and CHX mixture. PCA was not found in NaOCl and Qmix mixture.

was separated and dried over anhydrous sodium sulfate. Solvent was removed under reduced pressure, and a brownish solid (0.17 g) was obtained. This solid was used for TLC, 1H-NMR spectroscopy, GC, and HPLC analyses.

TLC

Ten mg brown solid was dissolved in 5 mL ethyl acetate. Two drops of this solution was injected on TLC plate. Near this spot, 2 drops of 10 mg solution of PCA in 5 mL ethyl acetate was injected as stationary phase. The plate was then placed in a TLC tank containing 1/2 (v/v) ethyl acetate/hexane mixture as mobile phase. TLC was run on foils (Fluka TLC-PET Foils; Sigma-Aldrich) with fluorescent indicator at 254 nm UV light (layer thickness, 0.2 mm; medium pore diameter 60 Å silica gel). Retention factor (Rf) for the brown precipitate and PCA was then calculated by dividing the distance traveled by the compound by the distance traveled by the solvent.

1H-NMR Spectroscopy

CHX solution was lyophilized for elimination of broad water signal from 1H-NMR spectrum. 1H-NMR spectra of lyophilized CHX, brown precipitate, and PCA were recorded in perdeuterated dimethyl sulfoxide separately in accordance with tetramethylsilane as an external standard in a 1H-NMR Spectroscopy Unit (Bruker UltraShield 500 plus; Bruker, Billerica, MA).

GC Analysis

Brown precipitate, PCA (as a standard), and mixture of brown precipitate with PCA (as an internal standard) were used. An HP-1 capillary column (25 × 0.32 mm inner diameter, 0.17-µm film thickness of cross-linked methyl siloxane) equipped gas chromatograph (Gas chromatograph, Agilent 6890 series; Agilent Technologies, Santa Clara, CA) was used. Oven temperature was set to 100°C for 2 minutes, and then it was increased to 280°C at a rate of 20°C/min and was maintained at this temperature for 10 minutes. The injector and the flame-ionization detector were maintained at 200°C and 250°C, respectively. Samples (1 mL) were introduced in a splitless mode (Agilent 7683 Series automatic injector; Agilent Technologies).

HPLC Analysis

Brown precipitate, PCA (as a standard), and mixture of brown precipitate with PCA (as an internal standard) were used for HPLC. HPLC analysis was performed by using conventional C18-column (150 m × 3.0 mm inner diameter) in flow rate of 0.5 mL/min under detection of diode array detector at 241 nm wavelength (Agilent 1200 SERIES HPLC System; Agilent Technologies) (22, 29, 30). Column oven temperature was set to 25°C, and injection volume was 20 µL. ACN and 1% phosphorous acid/water buffer were used for gradient elution as follows: 0–10 minutes 100% buffer, 10 minutes 50% ACN, 15 minutes 50% ACN, 17 minutes 100% buffer, 20 minutes 100% buffer. Each analysis was repeated 3 times by using 10 ppm solutions of sample and standard.

GC/MS Analysis

Two percent CHX was used for GC/MS. Methanol solution (1/10 v/v) of 2.0% CHX was injected to GC/MS unit (GC-MS, Agilent 5973 inert series, Mass Selective Detector System; Agilent Technologies) by using a capillary column (60 m length × 0.25 mm inner diameter and 0.25 mm film thickness) (Agilent HP-Innowax; Agilent

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