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Research Article

Saline Solution in the Formation of Para-Chloroaniline in the Reaction Between Chlorhexidine and Sodium Hypochlorite

María Carolina Bilbao Bravo[1], Silvana Maggiolo Villalobos[2], Miguel Neira Jara[3] and Ismael Yévenes López[4]*

[1]*Postgraduate Student in Dentistry, Faculty of Dentistry, University of Chile, Santiago, Chile*

[2]*Assistant Professor, Department of Conservative Dentistry, Faculty of Dentistry, University of Chile, Santiago, Chile*

[3]*Assistant Professor, Institute for Research in Dental Sciences, Faculty of Dentistry, University of Chile, Santiago, Chile*

[4]*Associate Professor, Institute for Research in Dental Sciences, Faculty of Dentistry, University of Chile, Santiago, Chile*

***Corresponding Author:** Ismael Yévenes López, Associate Professor, Institute for Research in Dental Sciences, Dental School, University of Chile, Sergio Livingstone 943, Independencia, Santiago, Chile.

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Abstract

Introduction: 5% sodium hypochlorite (NaOCl), saline (0.9% NaCl), and 2% chlorhexidine (CHX) forms a coloured precipitate para-chloroaniline (PCA). NaOCl dilution saline decreases the formation of precipitate but form a white precipitate.

Objective: The aim is to study the role of saline in the formation of PCA between NaOCl and CHX.

Material and Methods: 5% NaOCl diluted with 0.9% NaCl was mixed with 2% CHX to obtain PCA. NaOCl 0.05% and 0.005% were mixed with 0.9% NaCl and dilutions and 2% CHX, and PCA was quantified. 2% CHX was mixed with 0.9% NaCl and dilutions to form white precipitate. Spectrographic analysis of the precipitates were performed between hypochlorite 0.005%, saline with 2% CHX, and 0.9% NaCl with 2% CHX.

Results: Concentrated NaOCl solutions in 0.9% NaCl with 2% CHX formed PCA. Diluted hypochlorite forms a white precipitate. The formed PCA is directly related to concentrations of 0.005% and 0.05% NaOCl. 2% CHX formed precipitate only with 0.9% NaCl. The spectra of the precipitates formed by the reaction between 2% CHX with 0.9% NaCl and the reaction between 0.005% NaOCl with 2% CHX and 0.9% NaCl were equal. Their spectrographic analysis indicated chlorhexidine dihydrochloride.

Conclusions: In the reaction between chlorhexidine, sodium hypochlorite, and saline solution, two reactions were obtained: formation of PCA (NaOCl and CHX) and transformation of chlorhexidine digluconate into chlorhexidine dihydrochloride, which is responsible for the white precipitate. Until 0.5 % NaOCl, the first reaction predominates, and under this concentration, the second reaction begins. PCA measurements spectrophotometrically confirm this relationship.

Keywords: *Para-Chloroaniline; Chlorhexidine Digluconate; Sodium Hypochlorite; Chlorhexidine Dihydrochloride; Sodium Chloride; Absorption Spectrum*

Introduction

Generally, irrigants used in endodontic therapy are not completely eliminated from the root canal system (RCS) before using the following irrigant. Therefore, they come in contact with each other and can form solid products [4,14], which may occlude the dentinal tubules, forming a barrier that prevents proper sealing of RCS and increasing the risk of microfiltration [1,19]. In addition, these products can be toxic to the periapical tissues [5]. The simultaneous use of EDTA and NaOCl reduces the amount of hypochlorite, causing a decrease in the ability to dissolve tissue and antimicrobial activity, so such solutions should not be mixed [16,20]. By mixing EDTA and CHX, a pink precipitate appears and reduces the property of EDTA to remove the smear layer [7,16]. It has been indicated that the use of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate combined within the root canal resulted in the greatest percentage reduction of post-irrigant positive cultures [9]. However, if there is presence of NaOCl in the root canal when irrigated with CHX, a precipitate of orange-brown colour is formed and has been identified as para-chloroaniline (PCA) by some authors [19,20]; however, other authors have ruled out the presence of PCA in the precipitate using nuclear magnetic resonance spectroscopy (NMR), and they have identified it as parachlorophenylurea (PCU) and parachlorophenylguanidyl-1,6-diguanidyl-hexane (PCGH) [12,17].

In order to reduce precipitate formation, several solutions has been recommended as intermediate irrigators (e.g., saline solution, citric acid, or distilled water in large amounts) to cause a dilution effect on the NaOCl. However, none of them have been completely successful [11].

The saline solution has been little studied as an endodontic irrigant; however, it is widely used in the clinical and public sector in Chile and taught as part of the irrigation protocol in dentistry schools.

The interaction of 5% NaOCl with 0.9% NaCl and 2% chlorhexidine forms a p-chloroaniline (PCA) coloured precipitate. NaOCl dilution decreases the formation of the coloured precipitate, but the formation of a white precipitate is produced (Figure 1).

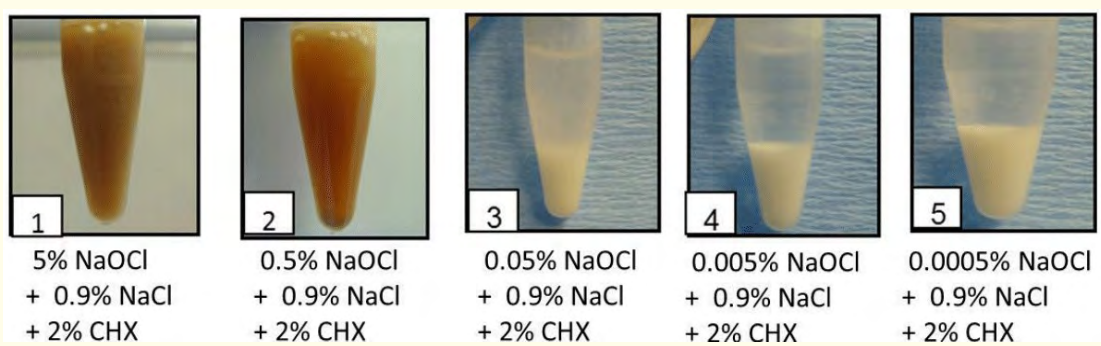

Figure 1: *Visual appearance of reactions between NaOCl dilutions in 0.9% NaCl plus 2% CHX.*

For saline solution as intermediate irrigant propose a dual role of dilute sodium hypochlorite and react with chlorhexidine digluconate. Today, there are no studies that show the mechanism of action of physiological saline in the interaction between sodium hypochlorite, chlorhexidine, and saline solution. Therefore, the aim of this study was to determine *in vitro* the mechanism of action of saline solution in the formation of para-chloroaniline (PCA) when used after 5% sodium hypochlorite and prior to 2% chlorhexidine digluconate.

Material and Methods

This is a prospective experimental and explanatory study *in vitro*. All chemical solutions were prepared by the chemistry laboratory at the faculty of dentistry based on the concentration requested.

λ_{max} determination and quantification of PCA

Dilution series (10^{-4} to 10^{-5} g/ml) from PCA standard concentration (0.39 g / 100 ml of PCA) (4-Chloroaniline. Sigma-Aldrich, St. Louis, MO., USA) were prepared. The absorption spectrum of the dilutions was registered in the UV-visible range (200-800 nm) in a spectrophotometer UNICAM®UV / VIS (Thermo Spectronic Unicam UV-530 UV-Visible, Rochester, NY, USA), using a quartz cuvette to determine the maximum lambda (λ_{max}). In order to relate absorbance with PCA concentration, a calibration curve was built with PCA standard solution of 0.00039%, 0.00078%, 0.000975% 0.00195%, and 0.039%. The measured absorbance of these solutions to λ_{max}, and a curve equation was determined to measure the concentration of PCA.

Reaction between 0.05% and 0.005% NaOCl dilutions in 0.9% NaCl with 2% CHX quantifying PCA formed

1 ml of 0.05% NaOCl and 1 ml of 0.005% NaOCl (Sigma-Aldrich 3050 Spruce St. St. Louis, MO 63103 USA) were reacted with 1 ml of 0.9% NaCl (Sodium chloride p.a. Sigma-Aldrich, St. Louis, MO., USA.) until 0.00009% NaCl dilution. They were mixed with 2 ml 2% CHX (Chlorhexidine digluconate solution 20% in H2O, Sigma-Aldrich 3050 Spruce St. St. Louis, MO 63103 USA). After 15 minutes, the absorbance of each mixture was measured in a spectrophotometer at λ_{max}, and the concentration of the PCA formed was measured using the equation of the calibration curve.

Reaction between 0.9% NaCl until 0.00009% NaCl dilutions and CHX 2%

1 ml of 0.9% NaCl until 0.00009% NaCl dilutions were mixed with 1 ml of 2% CHX. After 15 minutes, the result of the reaction was observed.

Identification by spectrophotometry of white precipitate formed in reactions between 0.005% NaOCl, 0.9% NaCl, and 2% CHX and between 0.9% NaCl and 2% CHX

The precipitate formed by reacting 1 ml of 0.005% NaOCl, 1 ml of 0.9% NaCl, and 2 ml of 2% CHX (Figure 1.3) and the precipitate formed by reacting 1 ml of 0.9% NaCl with 1 ml of 2% CHX were centrifuged at 10,000 rpm for 10 minutes in Hermle centrifuge Z216MK (HERMLE Labortechnik GmbH Siemensstrasse 25 78564 Wehingen). The residues were washed twice with distilled water and dissolved in 2 ml of methanol p.a. 10 mg of standard chlorhexidine dihydrochloride (Chlorhexidine dihydrochloride Sigma-Aldrich, St. Louis, MO., USA.) and were dissolved in 2 ml of methanol Sigma-Aldrich, St. Louis, MO., USA). Samples of each dissolution were transferred to Eppendorf tubes (1.6 ml) (Biologix Group Limited, No.2766 Ying XiuRoad, High-Tech Industrial Development Zone Jinan, Shandong 250101 P.R. China), centrifuged at 10,000 rpm for 5 minutes, and then the absorption spectrum was registered in the UV-visible range (200-800 nm) using a spectrophotometer UNICAM®UV / VIS (Thermo Spectronic Unicam UV-530 UV-Visible, Rochester, NY, USA) and a quartz cuvette.

Results

λ_{max} determination and quantification of PCA

The absorption spectra of the standard PCA indicated a λ_{max} of 375 nm. The calibration curve constructed with PCA concentrations of 0.039% to 0.00039 indicated a curve equation A = 638,75x [PCA] + 0.1864 and a coefficient of correlation between absorbance and PCA concentration of R^2 = 0.9981.

Reaction between 0.05% and 0.005% NaOCl solutions with 0.9% NaCl and 2% CHX quantifying PCA

Absorbance values to quantify the formation of PCA in the reaction between 0.05% and 0.005% NaClO with saline solution and dilutions with 2% CHX are shown in Figure 2. The absorbance values decrease as the concentration of saline solution decreases, and the measured absorbance values are much higher for the solution of 0.05% NaOCl compared to 0.005% NaOCl.

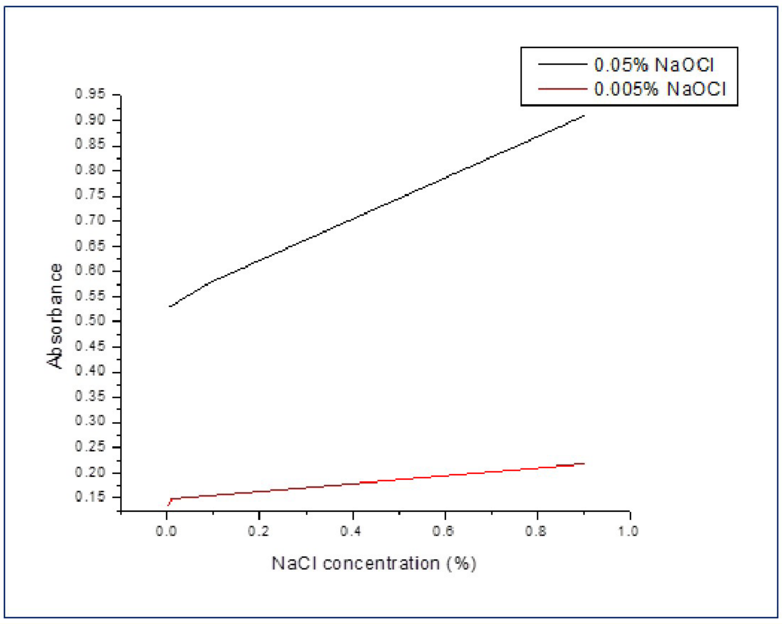

Figure 2: *Absorbance values for quantifying PCA from 0.05% and 0.005% NaClO at different concentrations of NaCl.*

A linear correlation is observed in the absorbance for formation of PCA for 0.05% NaOCl, which has an equation of formula: A= 0.4198 x [NaCl] + 0.5318 and a coefficient of correlation of 0.9994 (R^2).

For the 0.005% NaCl solution, a linear relationship is also obtained but with a lower slope. Its linear equation is A= 0.0835 x [NaCl] + 0.1424, and it has a correlation coefficient of 0.9723 (R^2).

Reaction between 0.9% NaCl until 0.00009% NaCl dilutions and 2% CHX

White precipitate formation was present only in the reaction between saline solution and 2% CHX. Higher dilutions of saline and 2% CHX showed no formation of precipitate.

Identification by spectrophotometry of white precipitate formed in reactions between 0.005% NaOCl, 0.9% NaCl, and 2% CHX and between 0.9% NaCl with 2% CHX. The spectral curves obtained from dissolutions of the precipitates formed in the reactions between 2% CHX with saline solution and 0.005% NaOCl, saline solution, and 2% CHX in methanol p.a. are displayed in Figure 3. As a standard, the spectral curve of chlorhexidine dihydrochloride was used. The spectrophotometric curves of the three alcoholic dispersions of solid forms present peaks within the range of wavelengths from 200 to 300 nm. The radiation in the UV spectrum causes electronic transitions to characteristic wavelengths of the molecular structure of the standard chlorhexidine dihydrochloride, resulting in a characteristic spectral curve. The spectral curves of the two precipitates analyzed are similar to the spectrum of the chlorhexidine dihydrochloride standard.

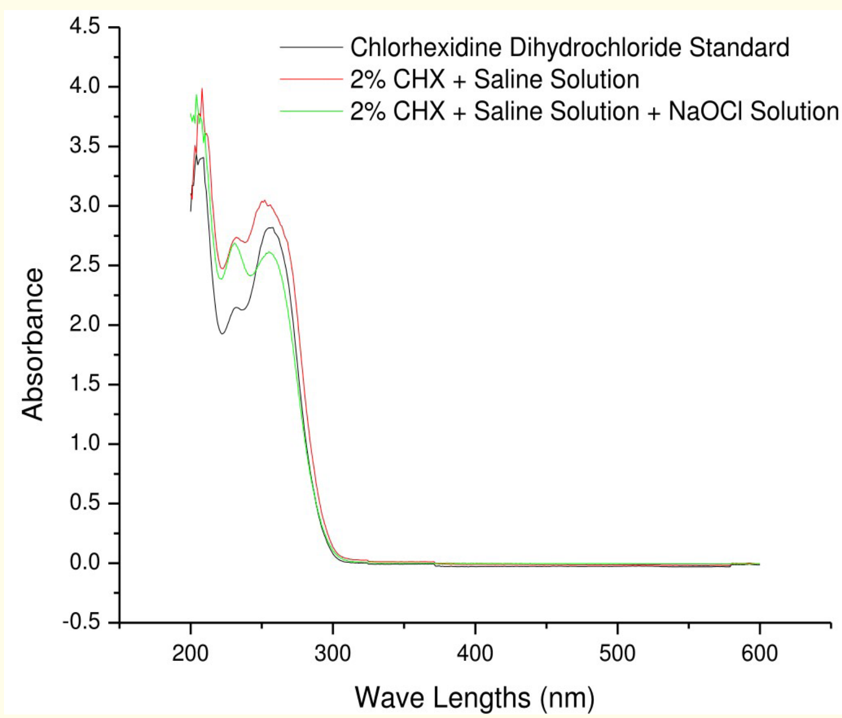

Figure 3: *: Spectral curves of solutions of the precipitates formed in the reaction between 2% CHX with 0.9% NaCl and between 0.005% NaOCl, 0.9% NaCl, and 2% CHX and the standard spectral curve of chlorhexidine dihydrochloride.*

Discussion

The main aim of this study was to determine *in vitro* the mechanism of the action of saline solution in the formation of para-chloroaniline(PCA) when used after 5% sodium hypochlorite and prior to 2% chlorhexidine digluconate.

Some studies have reported the occurrence of a color change and precipitation when NaOCl and CHX are combined [4,19]. Basrani., *et al*. [4] evaluated the chemical nature of this precipitate and reported that there was an immediate reaction when 2% CHX was combined with NaOCl. Even at low concentrations (0.023%), the product formed is para-chloroaniline (PCA).

The sequence of pictures in Figure 1 shows the chemical reactions that occur as the concentration of sodium hypochlorite decreases. Thus, in Figure 1.1, the reaction involves the 5% sodium hypochlorite in the presence of 0.9% NaCl and 2% CHX, forming the orange-brown precipitate characterized by Basrani., *et al*. as para-chloroaniline [4]. In Figure 1.2, NaOCl concentration decreases to 0.5% with 0.9% NaCl and 2% CHX, and a lighter orange-brown precipitate appears, corresponding with that indicated by Basrani., et al. who concluded that the precipitate is formed with hypochlorite concentrations up to 0.19% [4]. In Figure 1.3, the NaOCl concentration is 0.05%, NaCl is 0.9% and CHX is 2%. Formation of a white precipitate is observed, which coincides with the study reported by Prado., *et al.* who explains the formation of the precipitate between 2% CHX and saline solution as a salting-out process, i.e., the introduction of saline solution increased the concentration of salt and precipitated the CHX salts [2,13].

In Figures 1.4 and 1.5, NaOCl concentrations decreases to 0.005% and 0.0005%, while the other concentrations remain as 0.9% NaCl and 2% CHX. In both figures, the formation of a white precipitate is observed. With these concentrations of sodium hypochlorite in the presence of 2%CHX, the formation of a coloured solution of para-chloroaniline should be expected but is masked by the formation of white precipitate [15].

Looking at the photo sequence and chemical reactions involved, we hypothesize that there are two reactions that are simultaneously occurring. The first one indicates that the simultaneous presence of NaOCl and 2% CHX will lead to formation of para-chloroaniline with the presence of precipitate at high concentrations of NaClO but coloured solutions to more diluted concentrations [4,6]. The second reaction that we recognize in the photographic sequence is the conversion of chlorhexidine digluconate into chlorhexidine dihydrochloride, which is recognized qualitatively by the formation of white precipitate, i.e., the product of the reaction between 0.9% NaCl and 2% CHX [13]. The first reaction predominates at higher NaOCl concentrations, while the second one predominates at lower NaOCl concentrations.

To observe the influence of NaCl concentration (%) with 2% CHX, the saline solution and its dilutions were reacted with 2% CHX. Formation of white precipitate was observed only when 0.9% NaCl and 2% CHX were combined. Dilutions of physiological saline did not form precipitate in the presence of 2% CHX.

To visualize the effect of physiological saline dilutions in the formation of parachloroaniline, NaOCl was diluted to concentrations of 0.05% and 0.005% and reacted with saline solution and its dilutions as well as 2% CHX. As shown in Figure 2, the absorbance values that enable to quantify the formation of para-chloroaniline show similar curves for 0.05% and 0.005% NaOCl. In both curves, a direct dependency between the concentration of NaOCl and the absorbance value is observed. Both curves have positive slopes, and 0.05% NaClO has a greater slope. This is consistent with those reported by Basrani., *et al*. suggesting that the amount of PCA increases directly with the increasing concentration of NaOCl [3].

The quantification of the product formed using the calibration curve from standard PCA obtained a linear relationship that is steeper for higher concentrations of hypochlorite (5% and 0.5%), which confirmed the first reaction: formation of PCA from the reaction between hypochlorite and chlorhexidine.

The second reaction to postulate on the interaction between 5% sodium hypochlorite and its dilutions in presence of saline solution and 2%chlorhexidine digluconate is the conversion of a chlorhexine digluconate into chlorhexidine dihydrochloride. To test this second reaction, a UV-visible spectrophotometer was used. The spectrophotometric curves of the precipitates formed in the reaction between sodium hypochlorite, saline solution, and 2% CHX and between 2% CHX with saline solution were equivalent in size and shape to the spectrum obtained from a standard of chlorhexidine dihydrochloride. Assuming that electronic transitions that cause an absorption spectrum are dependent on the molecular structure, we can say that the standard of chlorhexidine dihydrochloride has a particular spectrum. Therefore, we suggest that similar spectra to the chlorhexidine dihydrochloride spectrum implies similar molecular structures. On this basis, we can deduce that the spectra obtained from the precipitates formed in the above reactions correspond to chlorhexidine dihydrochloride, confirming our second reaction.

The reaction between 5% and 0.5% NaOCl in presence of 0.9% NaCl and 2% CHX has been studied *ex-vivo* by Valera., *et al*. [18] who conclude that NaOCl irrigation, followed by irrigation with saline solution and final irrigation with CHX gel, produced the best root canal cleaning with the highest amount of open dentinal tubules. This was also studied by Krishnamurthy and Sudhakaran (2011), who concluded that the interaction between NaOCl and CHX resulted in an insoluble neutral salt as a precipitate that can be prevented using absolute alcohol and minimized using saline and distilled water as intermediate flushes [8].

The second reaction involving participation saline solution with 2% CHX has been studied by Marchesan., *et al*. [10], who found in their study that adding salt (NaCl) to chlorhexidine forms a white precipitate. Also, Prado., *et al*. [13] reported that the precipitates formed in the reaction of CHX with saline solution was associated with a salting-out process and lower solubility.

We could not find studies about the reaction involving NaClO concentrations of 0.05%, 0.005%, and 0.0005% with saline and CHX 2%.

Conclusions

When forming PCA between 5% NaOCl with 0.9% NaCl and 2% CHX, there are 2 reactions: first, there is the formation of PCA (NaOCl and CHX); second, there is a transformation of chlorhexidine digluconate to chlorhexidine dihydrochloride, which is responsible for the white precipitate formed. Up to 5 x 10-3% NaOCl, the first reaction predominates; under this concentration, the second reaction predominates. PCA measurements spectrophotometrically confirm this relationship.

Practical Implications

Avoid the simultaneous use of hypochlorite, saline, and chlorhexidine for the formation of precipitates that have not been studied in their clinical actions.

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Conflicts of Interest

The authors deny any conflicts of interest related to this study.

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