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Water and Physiological Saline to Prevent the Formation of P-Chloroaniline

Agua y Suero Fisiológico para Prevenir la Formación de Paracloroanilina

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ABSTRACT: This study determined if p-chloroaniline (PCA) can be minimized by using distilled water and physiological saline solution following sodium hypochlorite but before chlorhexidine. Hypochlorite 5%, 0.5%, 0.05%, 0.005% and 0.0005% dissolved in 0.9% NaCl and in distilled water were mixed with 2% chlorhexidine for the formation of PCA. The PCA was determined using UV-VISIBLE spectrometry, with spectral curve was determined the wavelength of maximum absorption of PCA. Formed PCA absorbance was measured between 0.025%, 0.02%, 0.015%, 0.01%, 0.005% and 0.0025% hypochlorite and 2% chlorhexidine. 2% chlorhexidine and hypochlorite with physiological saline form a white precipitate which prevents the measurement of PCA. Colored PCA is formed with 0.05%, 0.005% hypochlorite aqueous dilutions and 2% chlorhexidine. The lwavelength of maximum absorption obtained was 470 nm and absorbance of PCA showed a linear decrease. 0.005% NaClO produces the least amount of PCA. The best solvent to prevent the formation of PCA during the interaction of sodium hypochlorite with chlorhexidine is distilled water.

KEY WORDS: chlorhexidine; p-chloroaniline; distilled water; physiological saline solution; sodium hypochlorite.

INTRODUCTION

The objective in every endodontic treatment is to disinfect the whole root canal system before its obturation (Mohammadi, 2008). Studies have shown that conventional techniques of irrigation and implementation allow eliminating 90% of the bacterial colony. Ten percent of the remaining micro-organisms can proliferate between appointments reinfecting teeth at the same or at a greater extent than at the initial time of treatment (Siqueira, 2001). Therefore, it is necessary to use intracanal medication which increases treatment success rate from 68 to 95% according to Sjögren *et al*. (1997).

Many substances have been used for irrigation. However, and despite its permanence sodium hypochlorite has proven to be the chosen irrigator in modern practice due to its high tissue solution power, antibacterial and lubricant properties (Baumgartner & Cuenin, 1992).

Intracanal medication is an adjuvant in endodontic therapy and it consists in the placing of a drug into root canals at each appointment. This adjuvant becomes more important every day in Endodontics since it is known that even with a complete biomechanical preparation bacteria remaining in the root canal system (Almyroudi *et al*., 2002). New substances have been tested, at the moment 2% chlorhexidine (CHX) has proved to be the substance with better results, because at this concentration fungus *Candida albicans* (Basrani *et al*., 2004) and *Enterococcus faecalis* (Krithikadatta *et al*., 2007) can be eliminated, something that calcium hydroxide cannot do by itself. This was refuted by other authors, and accepted lately by Basrani *et al*. (2004) and reconfirmed by Krithikadatta *et al*. in 2007. The importance of CHX capacity to eliminate these two microorganisms is that there are always cases of rebel, refractory and persistent pathologies, and originating cases of endodontic therapy failure (Zerella *et al*., 2005).

The use of 2% CHX is also grounded in its effect over bacteria which are commonly found in teeth with endodontic pathologies, since it acts over anaerobic

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or facultative (Kontakiotis *et al*., 2008), positive and negative Gram, *i.e*., it is a wide spectrum acting antiseptic, even if it presents a lower action over negative Gram than over positive Gram (Davis *et al*., 2007; Haapasalo *et al*., 2010).

One of the negative aspects of CHX is its inactivation in presence of organic matter, also present in other irrigants / drugs used in endodontic treatment and that it is unable to remove organic tissue or smear layer. But this characteristic is not important to acting as medication, since this disadvantage of CHX is overcome thanks to the irrigation with sodium hypochlorite and the posterior use of EDTA.

Other negative aspect is CHX's interactions as it is known, CHX is harmless to the organism (Manzur *et al*., 2007; Wang *et al*., 2007; Rossi-Fedele *et al*., 2013); however its interactions can be harmful. Lately, it has been studied that there is a chemical reaction when using CHX to medicate the root canal of a tooth treated with sodium hypochlorite. This chemical reaction produces an orange substance. In this reaction an aromatic amine called para-chloroaniline (PCA) is released which has been proved to be toxic (Bui *et al*., 2008; Rasimick *et al*., 2008).

PCA is toxic by inhalation, digestion or skin contact. It has the capacity to produce sensitization at skin contact too. It has also been observed that it is able to produce cancer (World Health Organization, 2003; State of New Jersey Department of Health, 2015). It is included in the list of the IARC (International Agency for Research on Cancer, 2015) for highly carcinogenic substances to humans, along with other mono-chloroanilines.

In 2007 Basrrani *et al*. (2007) carried out a study about CHX and sodium hypochlorite interaction. This aimed to give importance to the compound formed by them. In this study the immediate reaction between these substances is shown, even when using low concentrations of hypochlorite (0.19%). As the hypochlorite concentrations rises, the precipitate increases in coloration and thickness, since this depends directly on sodium hypochlorite concentration (Bui *et al*.).

Inside the root canal, this precipitate affects the dentine's permeability, since at microscopic cuts dentinal tubules obliterated by PCA can be observed. This would imply sealing problems and, thus, it affects the treatment success (Prado *et al*., 2013). We do 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 between 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.

Currently, it is generally believed once the irrigation with hypochlorite is finished that the number of residual bacteria is responsible for endodontic failures. It can be controlled by placing an inter-appointment medication within the prepared canal, CHX and Calcium hydroxide, are the most commonly used inter-appointment dressings to wash the root canal with abundant saline solution or distilled water to thus dilute the sodium hypochlorite to the extent that PCA would not be formed when getting together with the CHX (Rahimi *et al*., 2014).

Based on the data from previous studies, it has been recommended to exercise caution and use an alternative irrigating solution between NaOCl and CHX to prevent the formation of this precipitate (Basrani *et al*., 2007; Prado *et al*.). Hence, the purpose of this study was to evaluate two commonly used endodontic solutions such as distilled water and physiological saline and their effects on preventing the formation of PCA using UV-VISIBLE spectrometry.

MATERIAL AND METHOD

Sodium Hypochlorite solution (Sigma-Aldrich, St. Louis, MO., USA), 4-Chloroaniline (Sigma-Aldrich, St. Louis, MO., USA), Chlorhexidine digluconate solution 20% in H_2O (Sigma-Aldrich, St. Louis, MO., USA), Sodium chloride p.a. (Sigma-Aldrich, St. Louis, MO., USA.). All chemical solutions were prepared by the chemistry laboratory at the faculty of dentistry based on the concentration requested. Solutions were then divided into two experimental groups of three tubes by dilution each and control of three tubes each. Tube type was identified and recorded appropriately.

Group 1. Hypochlorite dilution in 0.9% sodium chloride. The standard solution of 5% sodium hypochlorite was diluted successively with physiological serum solutions for 0.5%, 0.05%, 0.005% and 0.0005%. Five hundred milliliters of each dilution was taken and added to Eppendorf tubes. The procedure was repeated three times to obtain 15 samples. To each sample 500 mL 2% chlorhexidine was added.

Group 2. Hypochlorite dilution in distilled water. The standard solution of 5% sodium hypochlorite was diluted successively with distilled water dilutions for 0.5%, 0.05%, 0.005% and 0.0005%. Five hundred milliliters of each dilution was taken and added to Eppendorf tubes. The procedure was repeated three times to obtain 15 samples. To each sample 500 mL 2% chlorhexidine was added.

Group 3. Negative control. In three Eppendorf tubes with 500 mL of distilled water, 500 mL of 2% chlorhexidine was added.

Chemical measurement of the PCA compound. After the reaction was completed the solutions were prepared for analysis by UV-VISIBLE spectrometry. Spectrophotometric measurements were performed on spectrometer (Thermo SpectronicUnicam UV-530 UV-Visible, Rochester, NY, USA) obtaining absorption spectrum. From the absorption spectrum of the value of max is obtained when the PCA compound has the highest absorbance. This is used when making qualitative and quantitative determinations of the PCA compound. Three samples per tube were taken and were deposited in 96-well plate. The samples were taken to the visible light spectrophotometer aiming to determine the maximum compound absorbance of PCA. Using the wavelength of maximum absorption all samples were read in the spectrophotometer to determine the absorbance. Once the absorbance values were obtained they were written on a graph to evaluate the curve and to determine when the PCA compound stops forming. The final concentration of sodium hypochlorite in each dilution is also determined.

RESULTS AND DISCUSSION

Group 1. Hypochlorite dilution in 0.9% sodium chloride. Contact between NaOCl, CHX and sodium chloride produces a chemical reaction from which colored salt is formed. This colored salt is presented to the dilution 0.05% in NaOCl, from 0.005% dilution the solution is colorless, from this concentration a white precipitate appears. This salt clouds the mix making absorbance measurement not unreliable, this is the reason absorbance is not measured.

Group 2. Hypochlorite dilution in distilled water. Compounding p-chloroaniline is colored in the first 4 dilutions until the concentration of 0.005% in NaOCl. With this solution absorption spectrum was performed to obtain a wavelength of maximum absorption of 470 nm (Fig. 1). This graph allows determining the work wavelength. It was determined that the average –in accordance to the curve- is between 450 and 500 wavelengths. The absorbance average to the wavelength is fixed at 470.

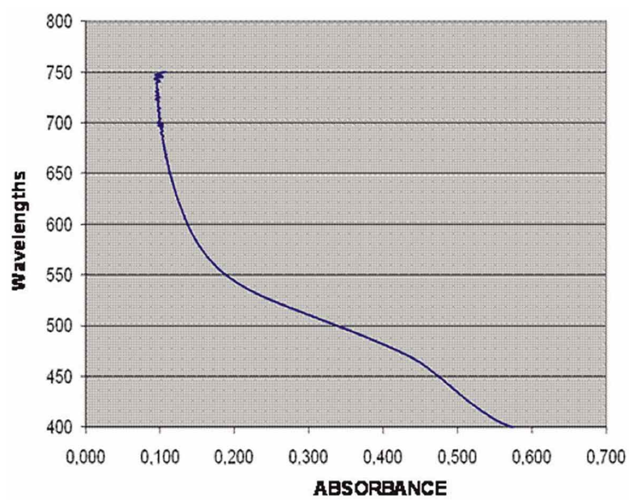

Fig. 1. Absorption spectrum of PCA, wavelengths (nm) curve versus absorbance and determination of max.

From the solution of 0.05% NaOCl, 2% CHX and distilled water dilutions with final concentrations of 0.025%, 0.020%, 0.015%, 0.010%, 0.005% and 0.0025% expressed as NaOCl we proceeded to measure the absorbance at lambda max of each of the dilutions. Once these values were known the absorbance versus PCA concentration expressed as sodium hypochlorite graph was built (Fig. 2).

Group 3. Negative control. Chlorhexidine at wavelength of 470 nm has residual absorbance values do not affect the other measurements in the presence of p-chloroaniline.

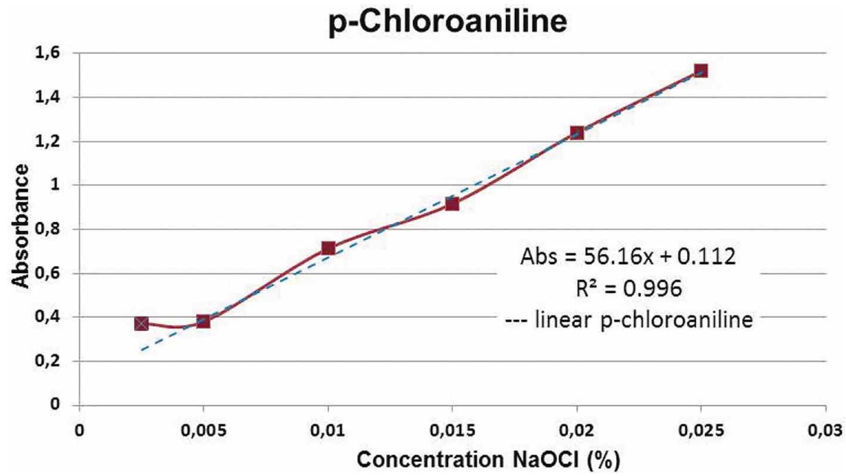

Fig. 2. Absorbance versus PCA concentration expressed as sodium hypochlorite. A linear decrease in absorbance was observed up to a concentration of NaOCl 0.005%, with a minimum in the formation of PCA.

It is well known that the combination of hypochlorite and CHX produces a colored precipitate called PCA which is toxic for the organism. This also happens within the tooth. Likewise, it has been observed that residues of PCA are difficult to remove from the walls of the root canal, affecting dentine's permeability, which can create problems with the sealing and posterior prediction of success in treatment (Bui *et al*.).

These are the reasons why two ways to dilute sodium hypochlorite for non-formation of PCA when is mixed with CHX were tried out.

Contact between 5% NaOCl and 2% CHX formed PCA with a strong orange coloration. This happens because the compounds are not diluted as they are used in dental office. The coloration slightly decreases due to hypochlorite dilution in saline solution (0.9% sodium chloride) and its concentration influences PCA formation directly. As the concentration of NaOCl decreases by dilution in physiological saline, the orange coloration decreases, a sign that PCA formation decreases but the formation of a white precipitate increases, showing an interaction between CHX and 0.9% NaCl. With this experiment we can tell that it is possible to decrease PCA formation with the dilution of hypochlorite in saline solution prior to the intracanal medication because no formation of colored precipitate is observed. However, the interaction between CHX and saline solution produces salt which without any doubt obliterates the dentinal tubules avoiding a proper contact between the sealing cement – wall and gutta-percha which decreases the possibilities of success in treatment. Saline solution diminishes PCA formation in bigger proportion than distilled water since it interacts with CHX forming salt and leaving less CHX to interact with sodium hypochlorite to produce PCA. The precipitates formed in the reaction of CHX with saline solution were associated with salting-out process, and lower solubility (Prado *et al*.).

Hypochlorite dissolves in distilled water and in the presence of CHX decreases strong orange coloration. The formation of PCA starts to linear decrease and in dilution of 0.005% in NaOCl practically no orange coloration is observed. No salt formation is observed since distilled water does not react with CHX or with sodium hypochlorite.

Measuring the absorbance determined that each of these dilutions was possible to find PCA and that this will always be produced while remnants of hypochlorite would exist in the mix. It is observed that the precipitate decreases considerably in color as sodium hypochlorite dilution increases, this is easily observed with unaided eye. PCA formation depends directly on sodium hypochlorite concentration, when shown on a graph the absorbance and link with PCA concentration expressed as sodium hypochlorite it is observed that starting from 0.005% concentration of sodium hypochlorite PCA formation starts to remain steady in the curve which indicates to us that from this dilution the minimum PCA can be obtained when contact with 2% CHX. It has been reported that Citric acid used as the intermittent irrigant had the least amount of PCA formation in the canal system. It can be hypothesized that positive results obtained with citric acid could be due to the partial release of the chlorine gas from the decomposition of NaOCl before the addition of CHX. However, just as with distilled water, this did not completely prevent the formation of PCA (Mortenson *et al*., 2012).

According to our results, the formation of p-chloroaniline (PCA), measured by absorbance values, decreases linearly as the concentration of sodium hypochlorite diminishes. This relationship is governed by the equation: Absorbance= 56.16 [NaOCl] + 0.112, with a very good correlation coefficient r= 0.992. This linearity (PCA formation and concentration of sodium hypochlorite) is maintained until the concentration of sodium hypochlorite 0.005%, with this concentration becoming minimum value in the formation of PCA. If this leads to clinical practice, and assuming that a residue arbitrarily 5% sodium hypochlorite after drying with paper tips and aspiration the 50 µl (0.05 mL), the volume that should be washed (dilute) the duct to bring the concentration of hypochlorite to its minimum (0.005%) is 50 mL.

Subsequently, after irrigating a root canal with 5% sodium hypochlorite it must be dried with paper tips and aspiration if it is needed to medicate with 2% CHX and if no PCA production is desired, compound which is toxic to the organism. The irrigation protocol used in the Faculty of Dentistry, Universidad of Chile, following the use of 10% EDTA, we proceeded to irrigate with saline solution with 3 mL three times. According to our results the serum of distilled water should be replaced, prior to use of CHX to 2%, because water does not react with CHX as well as serum forming a precipitate with 2% CHX. Until the actual volume of sodium hypochlorite remaining 5% without knowing the root canals, the total volume of irrigation should keep 3 syringes of 3 mL, with a total of 9 mL. This situation is different for those (Mortenson *et al*.) protocols where after the use of EDTA 5% sodium hypochlorite is reused, where the probability of finding residual hypochlorite posterior irrigation will be greatly increased and the greater will be the probability of forming PCA.

There are two studies that lead us to think it is the p-chloroaniline formed when reacting sodium hypochlorite and chlorhexidine. One notes that both XPS and TOF-SIMS showed the presence of p-chloroaniline in an amount directly related to the concentration of NaOCl used. Until this precipitate is studied further, its formation should be avoided by removing the NaOCl before placing CHX into the canal (Basrani *et al*., 2007). The other publication states: The TOF-SIMS results showed a peak at 127 mu, which is characteristic of 4-chloroaniline. However, this could also be characteristic of other isomers of 4-chloroaniline such as 2-chloroaniline and 3-chloroaniline. The results showed an absence of other aniline derivatives in the precipitate. Only PCA was found (Basrani *et al*., 2010).

Another aspect originated in our results is that the product formed in interaction with CHX hypochlorite is feasible to measure and has a linear response when p-chloroaniline is used as standard spectrophotometric determinations.

CONCLUSION

Saline solution diminishes PCA formation since it interacts with CHX, forming salt, leaving less CHX to interact with sodium hypochlorite to produce PCA. Distilled water diminishes PCA formation but it does not eliminate it completely since it only reduces sodium hypochlorite concentration. The best solvent of sodium hypochlorite to prevent the formation of PCA in contact with chlorhexidine is distilled water.

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RESUMEN: Este estudio determinó si la p-cloroanilina (PCA) puede ser minimizada mediante el uso de agua destilada y solución salina fisiológica seguido de la aplicación de hipoclorito de sodio, previo a la aplicación de clorhexidina. Hipoclorito al 5%, 0,5%, 0,05%, 0,005% y 0,0005% fue disuelto en 0,9% de NaCl y en agua destilada se mezcló con 2% de clorhexidina para la formación de PCA. El PCA se determinó mediante espectrometría UV-Visible, y con curva espectral se determinó la longitud de onda máxima del PCA. La absorbancia del PCA formado se midió con 0,025%, 0,02%, 0,015%, 0,01%, 0,005% y 0,0025% de hipoclorito y 2% de clorhexidina. La combinación de 2% de clorhexidina e hipoclorito en solución salina fisiológica forman un precipitado blanco que impide la medición del PCA. El PCA coloreado es formado con 0,05%, 0,005% diluciones acuosas de hipoclorito y 2% de clorhexidina. La longitud de onda máxima obtenida fue de 470 nm y la absorbancia del PCA mostró una disminución lineal. NaClO al 0,005% produce menor cantidad de PCA. El mejor disolvente para evitar la formación de PCA durante la interacción de hipoclorito de sodio con clorhexidina es agua destilada.

PALABRAS CLAVE: clorhexidina, p-cloroanilina, paracloroanilina, agua destilada, solución salina fisiológica, hipoclorito de sodio.

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