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Research Article

Distilled Water as an Intermediate Irrigant Between 5% Sodium Hypochlorite and 2% Chlorhexidine to Prevents the Formation of Parachloroaniline

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Abstract

Aim: To determine ex vivo if the use of distilled water as an intermediate irrigant between 5% NaOCl and 2% CHX prevents the formation of Parachloroaniline within the root canal.

Methodology: Thirty single-rooted non-carious human teeth extracted by therapeutic indication were selected and divided into a control and an experimental group. An endodontic treatment was applied to the teeth using the following irrigation protocol: 5% NaOCl from the access until the instrumentation was finished and then divided into a control group (n = 10) and an experimental group (n = 20). The control group was irrigated with saline (9 mL), followed by 10% EDTA (1 mL), then saline (9 mL) and a final irrigation with 2% CHX (3 mL). In the experimental group, the same irrigation sequence was followed but the saline was replaced by distilled water as an intermediate irrigant. The solutions of each root canal were collected during the different phases of the treatment using an intracanal aspiration device specially designed for this study. In the samples obtained, the concentration of NaOCl residual and Parachloroaniline was determined by spectrophotometry.

Results: A residual NaOCl concentration of 0.0029% was detected in the control group and 0.0025% in the experimental group after the use of the intermediate irrigant and prior to irrigation with 2%CHX. After irrigation with 2%CHX, a Parachloroaniline concentration of 0.00322% was measured in the control group and 0.00252% in the experimental group, with a statistically significant difference between the two groups.

Conclusions: The use of distilled water as an intermediate irrigant does not prevent the formation of PCA. PCA is formed within the root canal by the presence of residual NaOCl and 2%CHX. At least 9 mL of distilled water is required to dilute the 5% NaOCl sufficiently so that in contact with 2% CHX the minimum amount of PCA is formed

Keywords: *Irrigants; Sodium Hypochlorite; Chlorhexidine; Parachloroaniline; Spectrophotometry*

Abbreviations

CHX: Chlorhexidine; EDTA: Ethylenediaminetetraacetic acid; NaOCl: Sodium Hypochlorite; PCA: Parachloroaniline; λ_{max}: Maximum absorbance wavelength

Introduction

Because of the anatomic complexity of the root canal system, mechanical instrumentation cannot remove all the infected tissues and bacteria present in isthmuses and ramifications. Therefore, the use of irrigant solutions in association with mechanical instrumentation is required [1]. Irrigation in endodontics consists of the introduction of one or more solutions in the root canal system in order to remove vital or necrotic pulp tissue, dentin debris and microorganisms that can remain in the root canal even after adequate biomechanical preparation [2].

Unfortunately, there is no single irrigant that fulfills all the required functions, so optimal irrigation is based on the combined use of two or more substances used in a specific sequence to achieve therapeutic success [3].

When a combination of irrigants is used during endodontic therapy, the first irrigant used may not be completely flushed out from the root canal before applying the next one. As a result, endodontic irrigants routinely come into contact with each other inside the root canal and can form by-products [4,5], such as Parachloroaniline (PCA), a toxic precipitate formed by interacting Sodium Hypochlorite (NaOCl) and Chlorhexidine (CHX). This precipitate can occlude the dentinal tubules interfering with the cleaning and sealing of the root canal system, which makes the use of an intermediate irrigant essential to prevent its formation [6,7].

Prado*., et al.* [8] performed an *in vitro* study to characterize the by-products formed by associating irrigants most commonly used in endodontics: NaOCl at different concentrations (0.16% - 5.25%), 2%CHX, 17% EDTA, 10% citric acid, 37% phosphoric acid, saline, alcohol and distilled water. The results showed that when the NaOCl at different concentrations and the 2% CHX gel and solution were associated with distilled water, no precipitate formed. Thus, distilled water seems to be the best solution to be used as an intermediate irrigant to remove the remains of the previously used chemical.

The purpose of this research is to determine ex vivo whether the use of distilled water as an intermediate irrigant between 5% NaOCl and 2% CHX prevents the formation of Parachloroaniline within the root canal.

Materials and Methods

Thirty single-rooted non-carious human teeth extracted by therapeutic indication were selected for this study. The selection criteria of the samples were healthy uniradicular teeth with medium or wide canal caliber in the three-thirds of the root analyzed on the previous radiography. The specimens were stored in 0.9% saline until they were used. Soft tissues and calculus were removed mechanically from the root surfaces with a periodontal scaler. A size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was introduced, and the length was recorded when the tip of the file was visible at the apical foramen. The working length was determined by subtracting 1 mm from this length.

The apices of the specimens were sealed with wax to prevent overflow of irrigating solutions. Canals were instrumented using manual K-file instruments (Dentsply Maillefer) up to a size 35 file at the apex in a crown-down technique applying the following irrigation protocol: from the access cavity to finished instrumentation, the teeth were irrigated with 5% NaOCl (12 mL in total) using a Monoject Irrigation Syringe (size27-gauge needle) between each instrument change. The needle was placed at a distance of 1 mm from the working length, and it was moved backwards and forwards. The specimens were then divided randomly into a control group (n = 10) and an experimental group (n = 20). The control group was irrigated with 9 mL of saline, followed by 1 mL of 10% EDTA, then 9 mL of saline and a final irrigation with 3 mL of 2% CHX. In the experimental group, the same irrigation sequence was followed but the saline was replaced by distilled water as an intermediate irrigant. The solutions of each root canal were collected during the different phases of the treatment using an intracanal aspiration device specially designed for this study.

The samples collected by each tooth were transferred to Eppendorf tubes (1.5 mL) and centrifuged at 10,000 rpm for 5 minutes at 4°C, to remove residues and then proceeded to chemical analysis.

Determination of residual NaOCl concentration

In the samples collected during the treatment, the concentration of residual NaOCl that could remain inside the root canal after washing with the intermediate irrigant and prior to irrigation with 2% CHX was determined by spectrophotometry. For this, the wavelength at which the NaOCl has the highest absorbance was determined (λ_{max}): 292 nm (Figure 1). Then, the calibration curve was constructed and the equation of the curve was obtained, which allows to finally determine its concentration in the samples collected.

Subsequently, the absorbance at λ_{max} of the collected samples was measured on a spectrophotometer UNICAM®UV/VIS (ThermoSpectronicUnicam UV-530 UV-Visible, Rochester, NY, USA) using a quartz cuvette (1cc) against distilled white water and the absorbance values were interpolated into the equation of the calibration curve, to obtain the value of its concentration

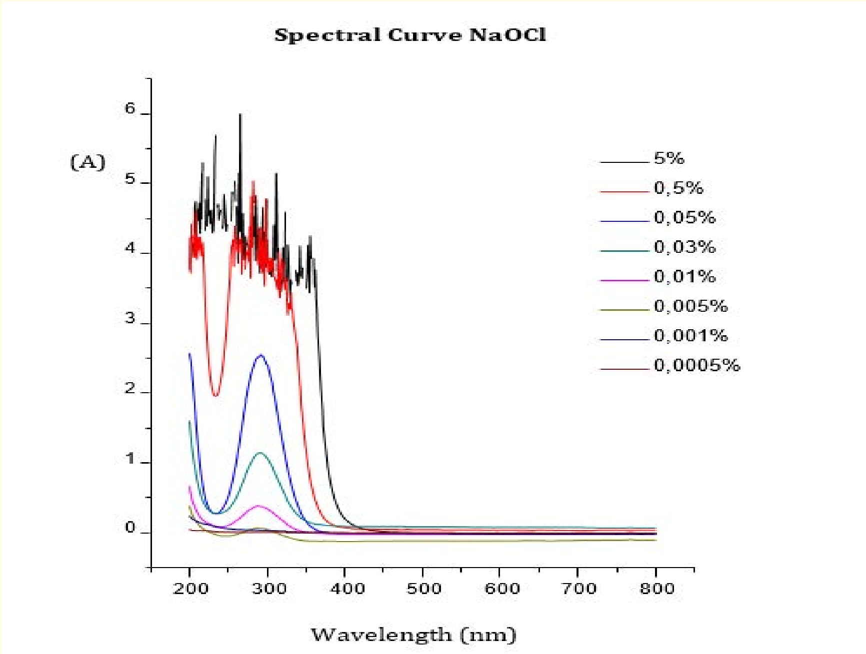

Figure 1: *Absorption spectra (Absorbance vs wavelength) of 5% NaOCl and dilutions at different concentrations. λ_{max}: 292 nm.*

Determination of absorption spectrum and PCA concentration

To determine the concentration of PCA in the samples, the protocol for the detection of Parachloroaniline in chlorhexidine digluconate solutions, described in the European Pharmacopoeia (2005) [9], was applied. 250 µL diluted Hydrochloric Acid (73 g/L) was added to 1mL of sample and diluted to 2 mL with distilled water. The following solutions were then added with shaking after each addition: 36 µL Sodium Nitrite (100g/L solution), 200 µL Ammonium Sulfamate (50 g/L solution), 500 µL N-(1-Naphthyl) ethylenediamine dihydrochloride (1 g/L solution), 100 µL Alcohol (Ethanol 96% solution). It was diluted to 5 mL with distilled water and allowed to stand for 30 minutes. The protocol was applied to 5 standards of PCA Aldrich® solutions of known concentrations and a change of coloration (red-blue) of different intensity according to PCA concentration was observed. Its absorbance was read in the visible range (350 - 800 nm) and its spectral curve was obtained to determine the λ_{max} (Figure 2). The calibration curve was constructed between 0,00005% and 0,001% of PCA and the curve equation was obtained, which allowed to determine the concentration of PCA in the samples collected.

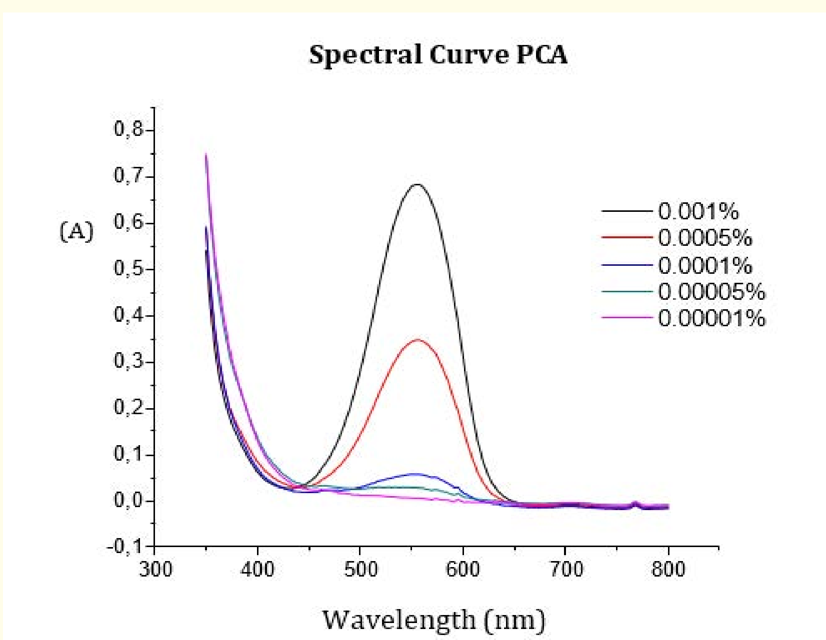

Figure 2: *Absorption spectra (Absorbance vs wavelength) of PCA solutions at different concentrations, after being submitted to the PCA identification protocol. λ_{max}: 552 nm.*

Statistical Analysis

The data obtained were subjected to the Shapiro-Wilk statistical test to determine the type of distribution. This test showed that the samples had no normal distribution. The data were then submitted to the Mann Whitney test to establish the significance of the differences found using the IBM SPSS statistical software. A 95% confidence interval was set, accepting statistically significant differences when $p < 0.05$.

Results

Using the spectrophotometric method, the residual NaOCl concentration after washing the root canal with the intermediate irrigant and prior to irrigation with 2% CHX was determined. The results obtained are shown in table 1. In both groups, it was possible to quantify NaOCl in small concentrations, slightly higher in the control group compared to the experimental group, but there is no statistically significant difference between the two groups.

Sodium Hypochlorite				
Control Group Saline		Experimental Group Distilled water		
Mean	Std. Deviation	Mean	Std. Deviation	p-value
0.00290%	0.00066	0.00250%	0.00099	> 0.05

Table 1: *Residual NaOCl concentration.*

Using the calibration curve prepared with PCA standards and the absorbance of the samples after being submitted to the PCA identification protocol, their concentration was determined in the last phase of endodontic treatment after the use of CHX 2% (Table 2). It is observed that there is a statistically significant difference ($p < 0.05$) in the measured PCA concentration, which is lower in the experimental group, where distilled water was used as an intermediate irrigant.

Parachloroaniline				
Control Group Saline		Experimental Group Distilled water		
Mean	Std. Deviation	Mean	Std. Deviation	p-value
0.00322% 32.2 ppm	0.00112 11.2	0.00245% 24.5 ppm	0.00064 6.4	< 0.05

Table 2: *Concentration of PCA.*

Discussion

Biomechanical cleaning and shaping of the root canals reduces the bacterial number but does not completely eliminate the bacteria from the canals and, therefore, requires the use of various irrigants in a sequential manner or in combination to enhance their antimicrobial effect. The combination of irrigants was shown to enhance their antimicrobial effect, and their interaction could be detrimental to the outcome of the root canal therapy [10]. The reaction between CHX and NaOCl is important because it results in a precipitate that includes PCA [4]. Thus, the aim of the present study was to determine *ex vivo* if the use of distilled water as an intermediate irrigant between 5% NaOCl and 2% CHX prevents the formation of PCA within the root canal.

The concentration of residual NaOCl remaining inside the root canal prior to irrigation with CHX 2% was measured. In both groups, it was possible to quantify NaOCl in low concentrations, slightly higher in the control group (0.0029%) compared to the experimental group (0.0025%), but there was no statistically significant difference between the two groups. Although these values are low, in the presence of 2% CHX they led to the formation of PCA. This is consistent with Bilbao [11] study about the influence of saline on the formation of PCA, when used between 5% NaOCl and 2% CHX, where it was shown that the dilution of NaOCl with saline does not prevent the formation of PCA. On the other hand, Riquelme., *et al.* [12] in an *in vitro* study performed a series of dilutions of NaOCl in distilled water, obtaining solutions of 0.025%, 0.02%, 0.015%, 0.01%, 0.005% and 0.0025% of NaOCl, which were mixed with 2% CHX. They determined that at each of these dilutions it was possible to find PCA and that this substance will always be produced as long as there are NaOCl remnants. The PCA values found are low (0.00322% and 0.00245%) but expressed in parts per million (ppm) are interesting values, for example 30 ppm of PCA could produce pharmacological and cellular changes.

Several studies have been carried out to evaluate the formation of PCA after using different solutions as intermediate irrigants between NaOCl and CHX. Krishnamurthy and Sudhakaran [10] evaluated the use of distilled water, saline and absolute alcohol to prevent PCA formation. The results showed that the only way to prevent the formation of PCA was intermediate irrigation with absolute alcohol. However, the interaction of CHX with alcohol produces saline precipitation, which could occlude the dentinal tubules. In addition, the use of absolute alcohol as an irrigant is not well established and the biocompatibility of this solution with the periapical tissues has not been fully studied.

Mortenson*., et al.* [7] evaluated whether it was possible to avoid PCA formation using saline, citric acid and EDTA as intermediate irrigants. However, the results showed that none of the solutions studied was able to prevent the formation of PCA.

A study by Magro., *et al.* [13] evaluated the efficacy of isopropyl alcohol, saline and distilled water to prevent the formation of precipitate due to the interaction between NaOCl and CHX and its effect on bond strength of an epoxy-based sealer in radicular dentine. The results showed that all of the solutions studied failed to prevent the precipitation of residues on canal walls following the use of NaOCl and CHX. However, these residues did not interfere with the push-out bond strength of the root filling. In contrast, Homayouni., *et al.* [14] showed that the presence of this precipitate does have a negative effect on the sealing capacity of the gutta-percha and the cement resin-based AH26 sealer, which is why they are recommended to prevent their formation using an intermediate irrigant between NaOCl and CHX.

In the present investigation, although the irrigation protocol proposed was different from those used in previous studies, PCA was also found, which could be due to the intermediate irrigants used (saline, distilled water and 10% EDTA), failed to eliminate completely the NaOCl remnants within the root canal system and, as long as these are present, it will always be possible to find PCA when later irrigated with 2% CHX. According to Riquelme., *et al.* [12], starting at a concentration of 0.005% of NaOCl, PCA formation begins to remain constant, indicating that from this dilution, the minimum amount of PCA can be obtained in contact with 2% CHX can be obtained.

In conclusion, none of the solutions used as an intermediate irrigant in this research could prevent the formation of PCA. Therefore, further studies are needed in order to find an irrigant that prevents the formation of this precipitate and that is biocompatible with the periapical tissues. For the moment, distilled water seems to be the best option as an intermediate irrigation solution, since it does not form by-products or precipitates when interacting with the other solutions used in the irrigation whereas the saline when interacting with the CHX produces a salt which can obliterate the dentinal tubules and alter the results of endodontic treatment. In addition, distilled water is innocuous to periapical tissues, and it can dilute the NaOCl to a concentration that allows a very low PCA formation being less than the one that is produced when using saline as an intermediate irrigant.

Conclusions

The use of distilled water as an intermediate irrigant allows to dilute the NaOCl within the root canal, but it does not prevent the formation of PCA, even though it is more effective than the saline in preventing the formation of this precipitate. PCA is formed within the root canal by the presence of residual NaOCl and 2% CHX. At least 9 mL of distilled water is required to dilute the 5% NaOCl to a concentration that upon contact with 2% CHX produces the least amount of PCA.

Conflict of Interest

The authors declare no conflicts of interest of any kind.

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