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Araştırma/ Research Article

SODIUM THIOSULPHATE AND TWEEN 80 PREVENT THE FORMATION OF PARA-CHLOROANILINE IN THE IRRIGATING SOLUTIONS

SODYUM TİYOSÜLFAT VE TWEEN 80 İRRİGASYON SOLÜSYONLARI İÇİNDE PARA-KLOROANİLİN FORMASYONUNU ENGELLER

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ABSTRACT
Aim: The aim of the present study was to evaluate the effect of using sodium thiosulphate and Tween 80 to prevent the formation of para-chloroaniline (PCA) between NaOCl and CHX in the apically extruded irrigating solutions.
Materials and Methods: Twenty-five extracted human mandibular premolar teeth were instrumented up to size 40. To collect the apically extruded irrigating solution, the roots were placed into the glass vial, and the specimens were divided into 5 groups according to the irrigation protocols, as follows; positive control, negative control, distilled water as intermediate flush between NaOCl and CHX, sodium thiosulphate irrigation between NaOCl and CHX, and Tween 80 irrigation between CHX and NaOCl. The collected liquid was centrifuged and the precipitate was analyzed using 1H NMR spectra. The data were analyzed with chi-square test at 95% confidence level.
Results: PCA was detected for all specimens in the positive control group. PCA was absent in the negative control group. Although distilled water irrigation did not prevent the formation of PCA, sodium thiosulphate and Tween 80 prevented the formation of PCA between NaOCl-CHX and CHX-NaOCl, respectively.
Conclusion: Within the limitations of the present study, sodium thiosulphate or Tween 80 prevented the formation of PCA between NaOCl-CHX and CHX-NaOCl, respectively.
Keywords: chlorhexidine, para chloroaniline, sodium thiosulphate

ÖZ
Amaç: Bu çalışmanın amacı NaOCl ve CHX arasında sodyum tiyosülfat ve Tween 80 kullanımının apikalden ekstrüze olan irrigasyon solüsyonları içinde PCA formasyonunun önlenmesine etkisinin değerlendirilmesidir.
Gereç ve Yöntem: Yirmi-beş çekilmiş mandibular premolar diş 40 numaraya kadar genişletildi. Apikalden ekstrüze olan irrigasyon solüsyonlarının toplanması için, kökler cam şişe içine yerleştirildi, örnekler irrigasyon protokolüne göre 5 gruba ayrıldı: pozitif kontrol, negatif kontrol, NaOCl ve CHX arasında distile su ile yıkama, NaOCl ve CHX arasında sodyum tiyosülfat ile yıkama, CHX ve NaOCl arasında Tween 80 ile yıkama. Toplanan sıvı santrifüje edildi ve çökelti 1H NMR spectra ile analiz edildi. Veriler ki-kare testi ile %95 güven aralığında analiz edildi.
Bulgular: Pozitif kontrol grubundaki tüm örneklerde PCA tespit edildi ve negatif gruptaki örneklerin hiçbirinde tespit edilmedi. Distile su irrigayonu PCA oluşumunu önlememesine rağmen, sodium tiyosülfat ve Tween 80 sırasıyla NaOCl -CHX ve CHX- NaOCl arasında kullanımla PCA formasyonunu engelledi.
Sonuç: Bu çalışmanın limitasyonları dahilinde, sodium tiyosülfat ve Tween 80, sırasıyla NaOCl -CHX ve CHX- NaOCl arasında kullanımla PCA formasyonunu engellemektedir.
Anahtar kelimeler: klorheksidin, para kloroanilin, sodyum tiyosülfat

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INTRODUCTION

One of the most important stages of the root canal treatment is the disinfection of the root canal system.[1] However, solely chemomechanical preparation is not sufficient for the complete disinfection of the root canal system having complexities in its internal anatomy.[2] Therefore, several antimicrobial irrigating solutions are used during preparation or as a final flush. Sodium hypochlorite (NaOCl) is widely used in root canal treatment because of its capacity of dissolving organic tissue and antimicrobial properties.[3-5] Despite of its advantages, NaOCl at high concentrations is toxic and it does not have antimicrobial substantivity.[6, 7] On the other hand, chlorhexidine (CHX) has a wide range of antimicrobial activity and its cationic structure provides antimicrobial substantivity.[8] Therefore, the combined use of NaOCl and CHX has been suggested.[9]

Since Vivacqua-Gomes et al.[10] realized the formation of orange-brown precipitate resulting from the combined use of CHX and NaOCl, a lot of studies have been conducted on the formation of precipitate,[11] detection of para-chloroaniline (PCA) in the precipitate,[12] and prevention of the precipitate or PCA formation.[13, 14] Bui et al.[11] evaluated the formation of precipitate on root dentin and dentinal tubules by using the environmental scanning electron microscope and found that the combination of NaOCl and CHX resulted in formation of precipitate tending to occlude the dentinal tubules. The PCA in the precipitate was first reported by Basrani et al.[12] The PCA was then detected in other studies by using gas chromatography-mass spectrometry,[13, 15] time-of-flight secondary ion mass spectrometry[16] or nuclear magnetic resonance (NMR) spectroscopy.[17] PCA in humans is a potent cyanogenic, carcinogenic,[18] toxic agent, and has immunotoxic effects.[19] As for precautions to prevent the formation of PCA, several irrigating solutions were used between NaOCl and CHX. Distilled water,[14] saline,[13, 14] isopropyl alcohol,[14] EDTA,[13] citric acid[13] were found to be insufficient to prevent PCA.

In previous reports sodium thiosulphate[20-22] and Tween 80[21, 23] have been extensively used to inhibit the antibacterial effects of NaOCl and CHX, respectively. However, there is no study in the literature evaluating the effect of using sodium thiosulphate and Tween 80 between NaOCl and CHX to prevent the formation of PCA. Thus, the aim of the present study was to evaluate the effect of using sodium thiosulphate and Tween 80 between NaOCl and CHX to prevent the formation of PCA in the apically extruded irrigating solutions. The null hypothesis was that there would be no differences between the groups.

MATERIALS AND METHODS

Twenty-five extracted human mandibular premolar teeth with similar dimensions were selected according to the following inclusion criteria: non-carious, fully formed apex, single root, single root canal, intact root without cracks or fractures, and a root canal curvature of < 10°. The extraction reasons of the teeth were not related to this study. The exclusion criteria were as following: previously root canal filled, internal/external resorption, and root canal calcification. To obtain a standardized length of 14 mm, the teeth were decoronated using a diamond disc.

A #10 K-file (Dentsply/Maillefer, Ballaigues, Switzerland) was inserted into the root canal. The length, at which the tip of file was visible at the apical foramen, was recorded and the working length was set as 1 mm less than this length. Root canal preparation was achieved with ProTaper Universal rotary instruments (Dentsply/Maillefer, Ballaigues, Switzerland) up to size 40. 2 mL of 5% NaOCl was used between each instrument change. To collect the apically extruded irrigating solution, the root was placed into the glass vial after silicone impression material was placed. The samples were randomly divided into 5 groups, according to the irrigation procedure, as follows:

Positive control: 5 mL of 5% NaOCl followed by 5 mL of 2% CHX,

Negative control: 5 mL of distilled water,

Distilled water: 5 mL of distilled water irrigation between 5% NaOCl and 2% CHX,

Sodium thiosulphate: 5 mL of 5% sodium thiosulphate (Meck, Darmstadt, Germany) irrigation between 5% NaOCl and 2% CHX,

Tween 80: 5 mL of 3% Tween 80 (Meck, Darmstadt, Germany) irrigation between 2% CHX and 5% NaOCl.

30 gauge side port endodontic needle with 25 mm length (Canal Clean, Biodent, Paju City, South Korea) was used for all irrigation procedures. The canals were then dried with paper points. The roots and silicone impression material were removed from the glass vials. The collected liquid was transferred to the microfuge tubes. The microfuge tubes were

centrifuged for 10 min at 7500 rpm and the photographs of the tubes were taken (Fig 1). The precipitate solids were removed, dissolved in d6-DMSO or CD_3OD solvents and analyzed using ^1H NMR spectra. The data were recorded as the presence or absence of the PCA and analyzed with chi-square test at the 95% confidence level ($P = .05$).

RESULTS

PCA was detected for all specimens in the positive control (Figure 1A and Figure 2A) and no PCA was formed in the negative control group (Figure 1B and Figure 3A). Distilled water irrigation between NaOCl and CHX did not prevent the formation of PCA (Figure 1C and Figure 2B). Sodium thiosulphate (Figure 2D and Figure 3B) and Tween 80 (Figure 1E and Figure 3C) prevented the formation of PCA between NaOCl-CHX and CHX-NaOCl, respectively ($P < .001$) (Table 1).

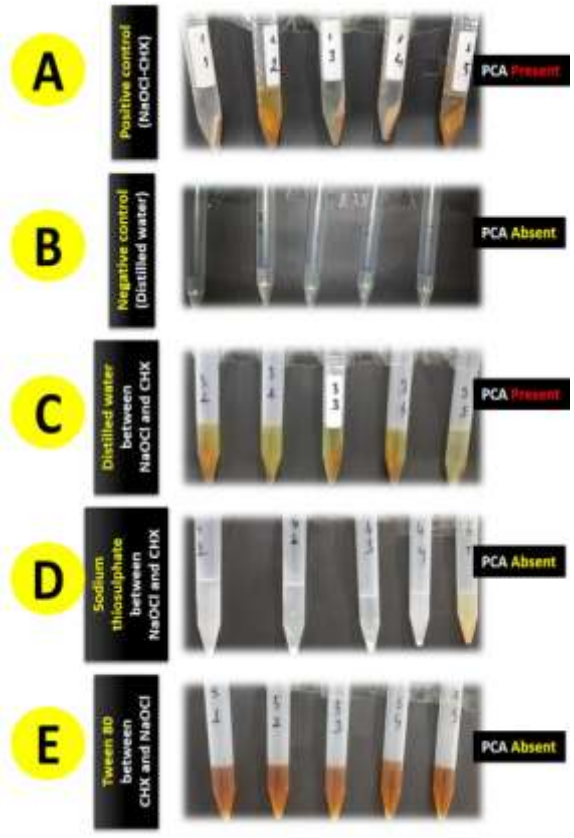

Figure 1. Images after centrifuge.

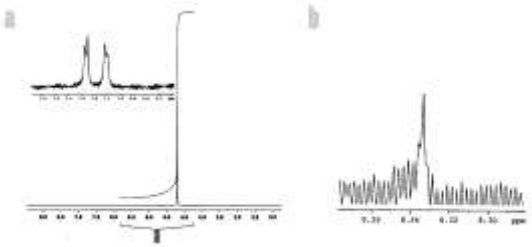

Figure 2. A peak between 7 and 8 ppm demonstrated the presence of para-chloroaniline. All of the spectra were obtained with a 400-MHz Bruker NMR System at 25 °C, acquiring 32 scans, in d6-DMSO solvent. Representative images for **a)** positive control (NaOCl-CHX) and **b)** distilled water between NaOCl and CHX.

Table 1. The number of specimens with PCA formation according to the groups. There were statistically significant differences among the groups ($P < .001$).

Groups	Para-Chloroaniline	
	Absent	Present
Positive control (NaOCl-CHX)	0	5
Negative control (Distilled water)	5	0
Distilled water between NaOCl and CHX	0	5
Sodium Thiosulphate between NaOCl and CHX	5	0
Tween 80 between CHX and NaOCl	5	0

DISCUSSION

Since the combined use of NaOCl and CHX has been suggested,[8, 9] there are several arguments related to the prevention of PCA.[13, 14] However, no tested irrigation solutions prevent the formation of PCA.[13, 14] In the literature, there are a lot of studies using sodium thiosulphate[20-22] or Tween 80[21, 23] to inhibit the effects of NaOCl and CHX, respectively. However, there is no study in the literature evaluating the effect of using sodium thiosulphate and Tween 80 between NaOCl and CHX to prevent the formation of PCA. Thus, the aim of the present study was to evaluate the effect of using sodium thiosulphate and Tween 80 between NaOCl and CHX to prevent the formation of PCA in the apically extruded irrigating solutions. According to the findings of the present study, there were statistically significant differences among the groups ($P < .001$). Thus, the null hypothesis was rejected.

Mortenson et al. [13] tested sterile saline, citric acid and EDTA to prevent the formation of PCA on root canals and found that although citric acid has the least amount of PCA formation in the canal system, none of the tested irrigating solutions prevented the formation of PCA in the canal system. Krishnamurthy

and Sudhakaran [24] evaluated the effectiveness of absolute alcohol, saline and distilled water to remove residual NaOCl and thereby prevent the formation of the precipitate, and found that PCA formation can be prevented using absolute alcohol and minimized using saline and distilled water as intermediate flushes. Krishnamurthy and Sudhakaran [24] also concluded that further investigation of the NaOCl/CHX precipitate in endodontic situations should address the bioavailability of PCA leaching out of the canal. In this sense, the present study evaluated the PCA formation in apically extruded irrigating solutions and revealed that distilled water was insufficient to prevent PCA formation in apically extruded irrigating solutions. However, sodium thiosulphate after NaOCl and Tween 80 after CHX prevent the formation of PCA. These results can be attributed to the inhibition effect of sodium thiosulphate on NaOCl[20-22] and the inhibition effect of Tween 80 on CHX. [21, 23]

PCA occludes the dentinal tubules and may compromise the seal of the obturated canal. PCA is cytotoxic, leading to the concern of it leaching out of the canal, and hence it has to be prevented or removed.[11, 15] Metri et al.[25] evaluated the effect of F-file and passive ultrasonic irrigation on removal of PCA and concluded that both of the techniques failed to completely remove PCA. Guneser et al.[26] compared different irrigation activation methods (conventional syringe, endodontic microbrush, sonic activation, photon-induced photoacustic streaming) in terms of removal of PCA and concluded that none of the techniques completely removed PCA from the root canals. Since it is difficult to remove PCA, it is very important to prevent it from occurring.

1H-NMR spectrum has been used in previous studies evaluating the formation of PCA.[17, 27] In the present study, apically extruded irrigating solutions was collected, centrifuged and then analyzed by using 1H-NMR spectrum. 1H-NMR spectrum exposes the specimens to a magnetic field and then to radio-frequencies that act on atomic nuclei.[27] Some authors found that NaOCl and CHX did not produce free PCA in any measurable quantity.[27, 28] However, in other studies the PCA was determined in the mixture of NaOCl and CHX.[11-13, 15-17] In the present study, PCA formation was determined in mixture of NaOCl and CHX collected from apically extruded irrigating solutions.

The present study revealed that sodium thiosulphate and Tween 80 can be beneficial as intermediate flush to prevent PCA formation. However, further studies should be conducted to confirm the findings of the present study.

CONCLUSION

Sodium thiosulphate inactivates NaOCl and Tween 80 inactivates CHX in apically extruded irrigating solutions. Within the limitation of the present study, sodium thiosulphate and Tween 80 prevents the formation of PCA between NaOCl-CHX and CHX-NaOCl, respectively.

NOT: Çalışmada herhangi bir yazar, kurum ya da kuruluş ile çıkar çatışması içerisinde bulunmamaktadır. Makale daha önce hiçbir yerde yayınlanmamış ve yayınlanmak üzere işlem görmemektedir

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