CHANGES IN pH OF IRRIGATING SOLUTIONS AFTER CONTACT WITH HUMAN ROOT DENTIN

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ABSTRACT

The aim of this study was to analyze the in vitro behavior of the pH of different irrigating solutions, used alone or consecutively, after contact with extracted human teeth. Mandibular human premolars were selected. The middle thirds were divided into 6 parts. The specimens obtained were divided into 6 groups and treated with irrigating solutions: 1) distilled water; 2) 1% NaOCl; 3) 1% Citric Acid (CA); 4) 17% EDTA; 5) 1% CA + 1% NaOCl; 6) 17% EDTA + 1% NaOCl. Specimens were immersed in 1 mL of each solution at 37ºC, those of groups 1, 2, 3 and 4, for 5 minutes, and the rest, consecutively for 2.5 minutes in each solution. Initial and final pH of the solutions were determined. Data were analyzed by the T Test, one-way analysis of variance (ANOVA) and Tukey multiple comparison Test. At 2.5 and 5 minutes there were significant differences between the initial and final pH for all solutions. The pH values decreased for distilled water and NaOCl, while they increased for CA and EDTA. In vitro, the pH of all solutions was modified after contact with root dentin at both test times (2.5 and 5 min).

Key words: *pH, irrigating solutions, dentin.*

VARIACIONES DEL pH DE SOLUCIONES DE IRRIGACIÓN ENDODÓNTICAS EN CONTACTO CON DENTINA RADICULAR HUMANA

RESUMEN

El objetivo de este trabajo fue estudiar in vitro el comportamiento del pH de diferentes soluciones de irrigación endodónticas, usadas solas o en forma consecutiva, después del contacto con dientes humanos extraídos. Se seleccionaron premolares inferiores. El tercio medio radicular se dividió en 6 partes. Los especímenes obtenidos se dividieron en 6 grupos, de acuerdo a la solución de irrigación empleada: 1) agua destilada; 2) NaClO 1%; 3) Ácido Cítrico 1% (AC); 4) EDTA 17%; 5) AC 1% + NaClO 1% ; 6) EDTA 17% + NaClO 1%. Los especímenes fueron sumergidos en 1 mL de cada solución a 37°C. Aquellos del grupo 1, 2 y 3 durante 5 minutos, y el resto, consecutivamente 2,5 minutos. Se determinaron pH inicial y final para cada solución. Los datos fueron analizados utilizando Test T, ANOVA y Test de comparaciones múltiples de Tukey. A los 2,5 y 5 minutos de exposición hubo diferencias estadísticamente significativas entre el pH inicial y final en todas las soluciones. El pH disminuyó en el caso de agua destilada e NaClO, mientras que aumentó en AC y EDTA. In vitro, el pH de todas las soluciones se modificó después del contacto con dentina radicular humana en ambos períodos de tiempo (2,5 y 5 minutos).

Palabras clave: *pH - irrigación - dentina.*

INTRODUCTION

Endodontic instrumentation produces a smear layer and plugs of organic and inorganic particles of calcified tissue and organic elements such as pulp tissue debris, odontoblastic processes, microorganism and blood cells in dentinal tubules[1]. Irrigation is considered the best method for removing tissue remnants and dentin debris during instrumentation[2,3]. Irrigating agents also provide lubrication, destruction of microbes and dissolution of tissues.

The efficiency of irrigating agents depends on root canal length, penetration depth of the substance, application time, dentin hardness, and concentration and pH of the solutions[2,4,5]. Because each solution is most effective at a specific pH, changes in pH value could modify its properties.

It has been suggested that chelating agents improve chemical-mechanical debridement in the root canal treatment by removing the smear layer from the root canal and demineralizing and softening dentin. The most commonly used chelating agents are based on different concentrations of ethylenediaminetetraacetic acid (EDTA) and citric acid (CA)[6,7]. Other non-chelating agents, such as sodium hypochlorite (NaOCl), have also widely been recommended as irrigants.

Initially, the use of EDTA solution was proposed by Ostby (1957) to assist with the instrumentation of calcified, narrow or blocked canals because of its ability to foster the chelation of the calcium ions at a pH close to neutral[8]. Its efficiency in removing inorganic dentin particles, preventing the formation of smear layer during instrumentation has been demonstrated[9-12]. It is used at 15-17% and pH 7-8.

CA, a weak organic acid, has a chelating demineralizing effect on calcified dentin components[13]. It has been previously applied on root surfaces altered by periodontal disease and flap surgery in order to increase cementogenesis and to accelerate healing, regeneration and normal periodontal attachment[14]. In operative dentistry, CA has been proposed as a mild etchant for hard dental tissues, particularly for dentinal conditioning, and enhanced smear layer and plug removal[6]. In endodontic treatments it is used at a concentration of 1%-50% and pH 0.8-1.9.

Fig. 1: Root dentin segments. A: coronal third; B, C and D: middle thirds; E: apical third.

Fig. 2: Root dentin middle third segments.

Fig. 3: Sections of root dentin middle third segments.

NaOCl has been widely recommended as an irrigant for chemical-mechanical debridement of root canals due to its solvent activity for necrotic and living tissues, in addition to its ability as an effective agent against broad spectrum bacteria[15-17]. It is used at a concentration of 1%-5.25% and at pH 11.9.

For maximum effect during and after instrumentation, chelating agents should be followed by tissue solvents. Alternating the use of EDTA or CA and NaOCl solutions has gained wide acceptance as an effective irrigation regimen[18-20].

The aim of this study was to evaluate *in vitro* the behavior of the pH of different irrigating solutions, used alone or consecutively, after contact with extracted human teeth.

MATERIAL AND METHODS

Experimental teeth and solutions

Ten recently extracted single-root human mandibular premolars were selected on the basis of their similarity in morphology and size. They were kept in distilled water at 4ºC until used. Debris, calculus and soft tissue remnants on the root surfaces were cleaned using a Gracey curette (Hu-Friedy, NC, USA). The crowns were sectioned at the cement-enamel junction using a high speed bur # 2200 (KG Sorensen, SP, Brazil) and water-irrigation. Cementum was removed using a Gracey curette. Root canals were enlarged up to a number 50 K-file (Maillefer, East Lansing, MI, USA),), at a working length of 1mm from the apex. They were cleaned and shaped using the step-back technique. After each instrument change, root canals were irrigated with 2 mL of distilled water, using a 25G needle (BD Precision Glide, Curitiba, Brazil). The apical and coronal third of the roots were removed and the remaining parts were cut transversally into three parts using a high speed bur # 2200 (KG Sorensen, SP, Brazil) (Fig. 1 and 2). Each slice was then bisected in buccolingual direction, obtaining a total of six sections of each root (Fig. 3). Sections of the same teeth were used to compare all the solutions. The sections were weighed on a precision scale (Acculab, BA, Argentina) (accuracy ≤0.1 mg) and found to have an average weight of 46.0 mg ± 13 mg. Then they were stored at 4ºC until use. The 60 specimens were divided into six experimental groups (ten specimens each) and treated with different irrigating solutions: group 1 (Control), distilled water (DW) pH 7; group 2, 1% NaOCl pH 11.6; group 3, 1% CA pH 1.8; group 4, 17% EDTA pH 7.2; group 5, 1% CA pH 1.2 + 1% NaOCl pH 11.6; group 6, 17% EDTA pH 7.2 + 1% NaOCl pH 11.6. The specimens in groups 1, 2, 3 and 4 were immersed in 1 mL of the irrigant at 37ºC for 5 minutes, and those in groups 5 and 6 were left in contact with 1 mL of each solution for 2.5 minutes resulting in a 5-minute immersion. Specimens were not washed between irrigants. All specimens were then removed and the pH of each solution was analyzed.

pH measurement

The pH of each solution was determined before and after contact with the dentin specimens using a digital pH meter (Broadley-Yames Corp. Irvine, Ca, USA) for small volumes (accuracy ≤0.01). The pH was determined by placing the refillable Calomel electrode in a 30 µL sample on a slide for 10 sec. The electrode was washed with distilled water and wiped dry between readings.

Statistical analysis

Data were analyzed using the T Test to compare the initial and final pH of each solution for related samples, and the final pH at different times for independent samples. Finally, one-way analysis of variance (ANOVA) was performed to compare the pH of the NaOCl solution when it was used alone or consecutively to CA or EDTA. Means were compared using the Tukey multiple comparison test.

RESULTS

Table 1 shows the pH values of the experimental solutions after contact with the sections of root dentin. At 5 minutes there were statistically significant differences ($p \leq 0.01$) between the initial and final pH values for all the solutions, including the control solution ($p \leq 0.05$). The pH values decreased for DW and NaOCl and increased for CA and EDTA.

When the irrigating solutions were used consecutively (Table 2), similar results were obtained: the pH values for DW and NaOCl decreased significantly ($p \leq 0.01$), while for CA and EDTA, they increased significantly ($p \leq 0.01$) after remaining in contact with the dentin for 2.5 minutes.

A comparison of the final pH values for DW, CA and EDTA solutions at both exposure times (5 minutes and 2.5 minutes) (Tables 1 and 2) showed no difference ($p \geq 0.05$) between the pH values of the DW and EDTA groups. However, CA showed statistically significant differences ($p \leq 0.05$) resulting in even higher pH values at 2.5 minutes than at 5 minutes contact time.

Regarding NaOCl solutions (Table 3), after the use of CA, and even more so with EDTA, pH was significantly lower ($p \leq 0.01$) at 2.5 minutes contact time compared to the pH value at 5 minutes.

Table 1: Initial and final pH of irrigating solutions after contact with human root dentin.

Solution	Time (min)	Initial pH (x ± SE)	Final pH (x ± SE)
DW	5	7.02 ± 0.00[a]	6.46 ± 0.13[b]
1% NaOCl	5	11.60 ± 0.00[a]	11.40 ± 0.00[b]
1% CA	5	1.80 ± 0.00[a]	2.00 ± 0.01[b]
17% EDTA	5	7.20 ± 0.00[a]	7.32 ± 0.02[b]

DW: Distilled water; NaOCl: Sodium hypochlorite; CA: Citric acid; EDTA: Ethylenediaminetetraacetic acid
*Significant differences are expressed by different letters (p≤0.05).

Table 2: Initial and final pH of consecutively used irrigating solutions after contact with human root dentin.

Solution	Time (min)	Initial pH (x ± SE)	Final pH (x ± SE)
DW	2.5	7.00 ± 0.00[a]	6.75 ± 0.08[b]
1% CA	2.5	1.80 ± 0.00[a]	2.11 ± 0.03[b]
1% NaOCl	2.5	11.60 ± 0.00[a]	11.30 ± 0.00[b]
17% EDTA	2.5	7.20 ± 0.00[a]	7.36 ± 0.02[b]
1% NaOCl	2.5	11.60 ± 0.00[a]	11.26 ± 0.01[b]

CA: Citric acid; NaOCl: Sodium hypochlorite;
EDTA: Ethylenediaminetetraacetic acid
*Significant differences are expressed by different letters (p≤0.05).

Table 3: Final pH of NaOCl solution alone and consecutively used after contact with human root dentin.

Solution (Time)	Final pH (x ± SE)
1% NaOCl (5 min)	11.40 ± 0.00[a]
1% NaOCl (2.5 min) after 1% CA (2.5 min)	11.30 ± 0.00[b]
1% NaOCl (2.5 min) after 17% EDTA (2.5 min)	11.26 ± 0.01[c]

NaOCl: Sodium hypochlorite; CA: Citric acid;
EDTA: Ethylenediaminetetraacetic acid
*Significant differences are expressed by different letters (p≤0.05)

DISCUSSION

The decalcifying action of CA, which has an acid pH, is greater than its chelating action, as reported in a paper by Machado-Silveiro *et al.* 2004[21] comparing CA to sodium citrate. They considered that sodium citrate may only have the chelating activity of the original acid, which is low and may explain why sodium citrate has lower decalcifying activity than CA.

De-Deus *et al.* 2006[22], reported that 10% CA caused peritubular and intertubular dentin erosion. Machado-Silveiro *et al.* 2004 also found stronger results with 1% and 10% CA than with 17% EDTA, while Spanó *et al.* 2009 contradict these results reporting that, when used for 5 min, 15% EDTA removed more calcium ions than 10% CA. Di Lenarda *et al.* 2000[23] found similar results for 1 $ml.L^{-1}$ CA and 15% EDTA. Haznedaroglu 2003[24] studied the effect of pH variation on the chelating effectiveness of CA, concluding that pH is a more important factor than concentration. These results are in agreement with Hennequin *et al.* 1994[8]. Thus, decalcification was higher with a CA solution at pH 1.1[9]. In addition to the pH variations of CA, EDTA and NaOCl with exposure time, we have demonstrated in other studies that these irrigating solutions did not significantly affect organic and inorganic human dentin composition at 2.5 minutes or 5 minutes exposure time[25].

Renewal of the solution increases the effectiveness of its action compared to a single continuous application over the same period of time[26] because it maintains the pH at natural levels, thereby increasing its moisturizing and decalcifying capacity[27]. Zehnder *et al.* 2005[28] reported that CA and EDTA may interfere with NaOCl action and should therefore be used separately. Both CA and EDTA immediately reduce the available chlorine in solution, rendering the sodium hypochlorite irrigant ineffective on bacteria and necrotic tissue. In our experience, NaOCl, like DW, did have lower pH after contact with the dentin, as if some acidic component of the exposed root tissue could be slightly sensitive to solubilization. On the other hand, CA and EDTA as chelating agents may act on the calcium dentin component, which may be responsible of the rise in the pH of the solution. However, exposure time might not affect the action of EDTA, as was demonstrated for CA, which had lower pH at 5 minutes than at 2.5 minutes, as if the dentin had shown buffering capacity. After the application of CA and EDTA, the solubilization effect of NaOCl may be much greater, since the dentinal tissue would be much more destabilized. However, it should be taken into consideration that cementum was absent from the root specimens in this experiment.

Dentin exposed to NaOCl may be sensitive to solubilization, an effect that may appear after the application of CA and EDTA, which may act on dentinal calcium. The exposure time used may not affect the pH of NaOCl and EDTA as it did for CA, which may provide evidence of dentinal buffering capacity at 5 min.

Further studies are needed to determine the pH behavior of the solutions, used alone and consecutively, in contact with human root dentin at higher exposure times.

This study should be complemented with others to determine biocompatibility of these drugs when used in endodontic treatments.

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