

Dissolution of Connective Tissue in Sodium Hypochlorite Alone and in Combination with 3% Hydrogen Peroxide

Disolución de Tejido Conectivo en Hipoclorito de Sodio solo y en Combinación con Peróxido de Hidrógeno al 3%.

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ABSTRACT: The aim was to evaluate the dissolution of porcine muscle using sodium hypochlorite in several concentrations and in combination with 3% hydrogen peroxide. Small pieces of porcine muscle were cut and their weight adjusted at 0.02g to be submerged in 1 ml of each test solution which was changed in 1 minute intervals until complete dissolution of the sample was observed. The 75 pieces of tissue were divided in 5 groups and different irrigant solutions were used as follows: Group 1, 5% sodium hypochlorite (NaOCl 5%). Group 2, 2.5% sodium hypochlorite (NaOCl 2.5%). Group 3, 2.5% sodium hypochlorite and 3% hydrogen peroxide alternately (NaOCl 2.5% + H₂O₂ 3%). Group 4, 3% hydrogen peroxide (H₂O₂), and group 5 used saline (NaCl 0.9%) as a negative control. For 5% sodium hypochlorite the mean time until complete dissolution of the sample was 17.5 minutes. The mean time for group 2 was 18.5, for group 3 was 64.6 minutes and for groups 4 and 5 there was no tissue dissolution after 120 minutes. To evaluate statistical significance ANOVA multi sample test was performed and Tukey post hoc test with significance level at P=0.05. Under the conditions of this study there were no significant differences with the use of sodium hypochlorite at 5% and 2.5% in terms of dissolution speed (P=0.6). The combination of sodium hypochlorite and hydrogen peroxide alternately caused a statistical significant delay in the dissolution of porcine muscle (P<0.05). The combination of sodium hypochlorite and hydrogen peroxide does not offer any benefit in terms of speed of dissolution of connective necrotic tissue.

KEY WORDS: sodium hypochlorite, irrigating solution, hydrogen peroxide.

INTRODUCTION

Chemo-mechanical preparation of the root canal is one of the main objectives of endodontic therapy (Hata *et al.*, 2001). Given the complex morphology of the root canal system, the achievement of this goal turns to be a challenge considering that endodontic files may contact only 35 to 53% of root canal walls, regardless of the instrumentation technique (Gulabivala *et al.*, 2005). Therefore, the use of irrigant solutions reaching inaccessible areas of the root canal anatomy for reducing debris and microorganisms becomes essential (Andersen *et al.*, 1992).

Endodontic irrigants clean and disinfect root canals by both, mechanical drag of microorganisms and debris and by chemical action dissolving cell membranes denaturalizing proteins and inactivating microbial toxins (Cohen & Burns, 2004). Sodium hypochlorite (NaOCl) and hydrogen peroxide (H₂O₂)

have been widely used as irrigants for root canal therapy being the first one the most important and irreplaceable because of its properties including tissue dissolving capacity and antimicrobial effect (Naenni *et al.*, 2004). These desirable characteristics come mainly from oxidizing and hydrolyzing cell proteins, releasing chloride to form hypochlorous acid and its osmotic potential to extract liquids out of the cell (Di Lenarda *et al.*, 2000).

On the other hand, hydrogen peroxide has disinfecting properties and it is used for root canal therapy as an irrigant solution in a concentration of 3%. Its action mechanism comes by liberation of oxygen which is toxic for anaerobic microorganisms and by an effervescent reaction produced once it makes contact with organic tissue and certain chemical compounds. This reaction has been proposed to aid in the removal of debris from root canal walls (Ohara *et al.*, 1993).

Combined use of these irrigants (NaOCl and H₂O₂) is however, a matter of controversy. Grossman *et al.*, (1988) indicate that the benefits from this combination are: a) effervescent reaction that produces removal of debris from root canal, b) dissolving capacity of organic tissue by sodium hypochlorite and c) disinfecting and bleaching action from both agents. Nevertheless, Baumgarther & Mader (1987) report that sodium hypochlorite is more effective alone than in combination with hydrogen peroxide.

In this *in vitro* study the dissolution of connective tissue by the action of these irrigants was evaluated, both alone and combined, to determine if the speed of dissolution was affected.

MATERIAL AND METHOD

Seventy five pieces of fresh porcine muscle were cut at room temperature and their weight adjusted to 0.02 g with an analytic bascule (AS220/c/2, Radwag, Croatia, Poland) to be divided into five experimental groups.

A series of 120 Dappen glasses were prepared containing 1 ml of an irrigating solution according to the experimental group assigned. For group 1, all the glasses contained 5% sodium hypochlorite. Group 2 glasses were filled with 2.5% NaOCl. In group 3 glasses were filled with either 2.5% NaOCl or 3% H₂O₂ and placed in alternated manner, with the first one at 2.5% NaOCl. For group 4 all glasses contained H₂O₂. Group 5 used saline (0.9% NaCl) as negative control.

Every sample of tissue was then submerged in the test solution for a period of 1 minute (Fig. 1) then removed and immediately submerged in the next glass for the same period of time. This procedure was repeated until complete dissolution of the sample was observed and time passed was taken. For easier manipulation of the sample, it was pierced with an atraumatic half circle 5-0 suture needle.

The immersion time of the sample within the test solution was measured with an automatic recurring timer (G-8000G, Casio, Miami, E.E.U.U).

Statistical differences between groups were evaluated with ANOVA test for multiple measurements and Turkey's post hoc. P value of P<0.05 was considered statistically significant.



Fig. 1. Sample tissue immersed in one of the irrigants.

RESULTS

Time required for complete dissolution of the sample was recorded for each group and the mean is shown in Table I. Group 1 (5% NaOCl) was the fastest in dissolving the samples with a mean of 17.5 minutes. Group 2 was slower than 1 although the difference was not significant (P=0.6). Time required for dissolution of samples in group 3 was significantly longer than that for groups 1 and 2 (P<0.05) as shown in Figure 2 and Table I. Samples in group 4 turned into a white color after successive immersions in 3% H₂O₂ but there was no tissue dissolution at all after 120 minutes. Group 5 didn't show either change or dissolution of the samples

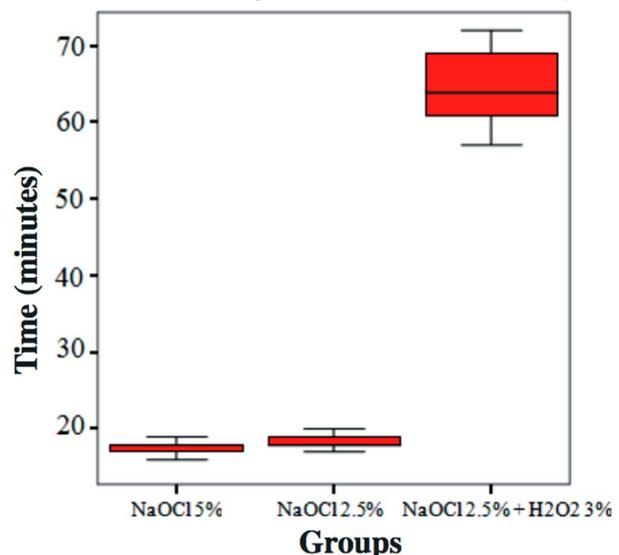


Fig. 2. Mean time until complete dissolution of muscular porcine tissue for groups 1, 2 and 3.

Table I. Mean time until complete dissolution of the test sample.

Group	Irrigant solution	Time in minutes (*)
1	NaOCl 5%	17.5(a)
2	NaOCl 2.5%	18.5(a)
3	NaOCl 2.5% + H ₂ O ₂ 3%	64.6(b)
4	H ₂ O ₂ 3%	> 120(c)
5	Saline	> 120(c)

(*) Different letters show statistical significant differences ($p < 0.05$).

DISCUSSION

Under the parameters of this study, the combination of sodium hypochlorite and hydrogen peroxide did not offer any benefit in terms of speed of dissolution of connective necrotic tissue. When the test sample from group 3 (2.5% NaOCl alternated with 3% H₂O₂) was immersed in each of the irrigants, a strong effervescent reaction was observed around the specimen. However, it did not cause the disruption or disintegration of this organic tissue.

Comparing the time required for complete dissolution of connective tissue with sodium hypochlorite in different concentrations it was found that although 5% NaOCl worked faster (Mean time 17.5 min) the difference with 2.5% was not statistically significant (Mean time 18.5 min) ($P=0.6$).

Caution must be taken when extrapolating data from in vitro studies. Factors like temperature, pH, of the irrigant solution and buffer capacity of dentine may interfere with the effect of sodium hypochlorite in clinical environment (Hilgren *et al.*, 2007; Cristensen *et al.*, 2008) and they were not considered in this study. Furthermore, the test samples were completely immersed in the irrigating test solution which was exchanged every minute, something impossible to achieve during root canal therapy (Hasselgren *et al.*, 1988).

Porcine muscular tissue has been previously used when evaluating sodium hypochlorite capability to dissolve necrotic connective tissue showing interesting results (Hasselgren *et al.*; Clarkson *et al.*, 2006; Christensen *et al.*). Even though it has obvious histological differences with human pulp tissue, it provides an easy manipulation and standardization of the samples and also reproducible results.

The combination of sodium hypochlorite and hydrogen peroxide has been proposed for irrigation of root canals because it produces a transitory but strong effervescent reaction that is assumed should aid in the removal of debris and micro organisms from within the root canal (Shiozawa, 2000).

Shiozawa studying the chemical reaction between these two irrigating solutions found that the addition of hydrogen peroxide to sodium hypochlorite produces bubbles due to decomposition of H₂O₂ and the oxygen (O₂) release. This reaction decreases the NaOCl concentration making tissue dissolution less effective. The same author emphasizes that NaOCl toxicity for periapical tissues along with the potential damage caused by O₂ release constitute a mayor problem when this irrigant combination is applied. The results from our study make it clear that the effervescence produced by this chemical reaction does not improve the dissolution capability of NaOCl in terms of necrotic connective tissue dissolution speed.

Although the combination of NaOCl and H₂O₂ has been used in clinical practice, evidence suggests that there is no benefit in the dissolution of connective tissue (Baumgarther & Mader; Shiozawa), and considering the potential chemical irritation for periapical tissues added to the risk of causing emphysema (de Sermeño *et al.*, 2009) it might seem reasonable to disregard the alternating use of these irrigants.

Toxicity of NaOCl is directly related with its concentration (de Sermeño *et al.*). Under the parameters of this study 5% NaOCl was not significantly faster than 2.5 % ($P=0.6$) in dissolving necrotic tissue. Therefore, 2.5% concentration could be considered as a good alternative for an irrigating solution with less toxicity and still good dissolving properties (Sánchez *et al.*, 2009).

In conclusion, combination of 2.5% NaOCl and 3% H₂O₂ significantly delays the dissolution of necrotic muscle tissue and we do not recommend its use. 5% NaOCl was the fastest irrigant solution in dissolving the test samples; nevertheless, 2.5% NaOCl has lower irritation potential for periapical tissues and the difference in dissolution speed lacked statistical significance.

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RESUMEN: El objetivo fue evaluar la disolución de tejido muscular porcino por acción del hipoclorito de sodio en varias concentraciones y en combinación con peróxido de hidrógeno al 3%. Se cortaron trozos de tejido muscular porcino y se ajustó su peso a 0,02 g para ser inmersos en 1 ml de cada una de las soluciones de irrigación de prueba hasta observar la total disolución de la muestra. 75 piezas de tejido fueron divididas en 5 grupos y se utilizaron diferentes soluciones de irrigación como se describe: Grupo 1, hipoclorito de sodio al 5% (NaOCl 5%). Grupo 2, hipoclorito de sodio al 2,5% (NaOCl 2,5%). Grupo 3, hipoclorito de sodio al 2,5% alternado con peróxido de hidrógeno al 3% (NaOCl 2,5% + H₂O₂ 3%). Grupo 4, peróxido de hidrógeno al 3% (H₂O₂) y el grupo 5 utilizó solución salina (NaCl 0,9%) como control negativo. Para el hipoclorito de sodio al 5% la mediana del tiempo hasta la disolución total del tejido fue 17,5 minutos. La mediana del tiempo para el grupo 2 fue 18,5 para el grupo 3, 64,6 minutos y para los grupos 4 y 5 no se observó disolución del tejido de muestra tras 120 minutos. La significancia estadística se determinó con el test ANOVA multi muestra y se realizó test Tukey pos hoc con un nivel de significancia P=0,05. Bajo los parámetros de este estudio no hubo diferencia significativa con el uso de hipoclorito de sodio al 2,5 y 5% en términos de velocidad de disolución del tejido conectivo (P=0,6). La combinación de hipoclorito de sodio y peróxido de hidrógeno de forma alternada causó un retraso estadísticamente significativo en la disolución de tejido muscular porcino (P<0,05). El uso alternado de hipoclorito de sodio y peróxido de hidrógeno no ofrece ningún beneficio en términos de velocidad de disolución de tejido conectivo necrótico.

PALABRAS CLAVE: hipoclorito de sodio, soluciones de irrigación, peróxido de hidrógeno.

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