Revista Odonto Ciência
Journal of Dental Science

ORIGINAL ARTICLE

Rev Odonto Cienc 2016;31(3):109-113
http://revistaseletronicas.pucrs.br/ojs/index.php/fo
http://dx.doi.org/10.15448/1980-6523.2016.3.21745

Open Access

Effectiveness of calcium and sodium hypochlorite associated with passive ultrasonic irrigation on pulp tissue dissolution – an *in vitro* study

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ABSTRACT

Objectives: To compare, *in vitro*, the effectiveness of calcium hypochlorite and sodium hypochlorite associated with passive ultrasonic irrigation (PUI) on pulpal tissue dissolution.

Methods: The pulp tissue of forty-five bovine incisors was removed and each complete pulp was divided into two pieces of similar volume, resulting in 90 pieces distributed into nine groups (n=10), according to dissolution procedure: G1: distilled water; G2: 2.5% NaOCl; G3: 5.25% NaOCl; G4: 2.5% $Ca(OCl)_2$; G5: 5.25% $Ca(OCl)_2$; G6: 2.5% NaOCl+PUI; G7: 5.25% NaOCl+PUI; G8: 2.5% $Ca(OCl)_2$+PUI and G9: 5.25% $Ca(OCl)_2$+PUI. Each sample was weighed on a high precision balance before and after the dissolution procedure. The differences in weights were calculated and the mean percentage loss was determined. The data were statistically analyzed using two-way ANOVA and Games-Howel tests at 5% significance level.

Results: Group 5 (5.25% $Ca(OCl)_2$), 6 (2.5% NaOCl+PUI), 7 (5.25% NaOCl+PUI), 8 (2.5% $Ca(OCl)_2$+PUI) and 9 (5.25% $Ca(OCl)_2$+PUI) showed the highest mean percentages of weight reduction when compared to other groups ($p<0.001$). However, there was no statistically significant difference between them ($p<0.001$).

Conclusions: $Ca(OCl)_2$ and PUI contributes significantly to dissolution of pulp tissue.

Key words: Calcium hypochlorite; Passive ultrasonic irrigation; Pulp dissolution; Sodium hypochlorite

Eficácia do hipoclorito de cálcio e de sódio associado com irrigação passiva ultra-sônica na dissolução do tecido pulpar – um estudo *in vitro*

RESUMO

Objetivo: Comparar, *in vitro*, a eficácia do hipoclorito de cálcio ($Ca(OCl)_2$) e hipoclorito de sódio (NaOCl) associados com irrigação passiva ultra-sônica (PUI) na dissolução de tecido pulpar.

Metodologia: O tecido pulpar de quarenta e cinco incisivos bovinos foi removido e dividido em duas peças de volume similar, resultando em 90 amostras distribuídas em nove grupos (n=10), de acordo com o procedimento de dissolução: G1: água destilada; G2: NaOCl 2,5%; G3: NaOCl 5,25%; G4: $Ca(OCl)_2$ 2,5%; G5: $Ca(OCl)_2$ 5,25%; G6: NaOCl 2,5%+PUI; G7: NaOCl 5,25%+PUI; G8: $Ca(OCl)_2$ 2,5%+PUI; G9: $Ca(OCl)_2$ 5,25%+PUI. Cada amostra foi pesada em uma balança de alta precisão antes e após o procedimento de dissolução. As diferenças em pesos foram calculadas e a média de perda percentual foi determinada. Os dados foram analisados estatisticamente utilizando two-way ANOVA e Games-Howel test (α=5%).

Resultados: Grupo 5 ($Ca(OCl)_2$ 5.25%), 6 (NaOCl 2.5%+PUI), 7 (NaOCl 5.25%+PUI), 8 ($Ca(OCl)_2$ 2.5%+PUI) e 9 ($Ca(OCl)_2$ 5.25%+PUI) apresentaram as mais elevadas médias percentuais de redução de peso quando comparados aos demais grupos ($p<0.001$). No entanto, não houve diferença estatisticamente significante entre eles ($p<0.001$).

Conclusão: $Ca(OCl)_2$ and PUI contribuem significativamente para a dissolução de tecido pulpar.

Palavras-chave: Dissolução pulpar; Hipoclorito de cálcio; Hipoclorito de sódio; Irrigação passiva ultra-sônica

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Received: August 30, 2105
Accepted: October 23, 2016

Conflict of Interests: The authors state that there are no financial and personal conflicts of interest that could have inappropriately influenced their work.

INTRODUCTION

The main etiological factors that can lead to failure of endodontic therapy are the persistence of microorganisms and residual pulpal tissue in the root canal system; this exerts a significant role in the progression of periapical pathologies [1]. Therefore, the use of irrigant solutions with ability to dissolve pulp, especially from inaccessible areas, contributes significantly to debridement of root canal space, since the removal of pulp tissue is inadequate with mechanical instrumentation alone, due to the complexities of the anatomical root canal system [2].

Several studies have been conducted to search for an irrigant to provide four major properties: antimicrobial activity, non-toxicity to periapical tissues, solubility in water and ability to dissolve organic matter. Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant during the chemomechanical debridement of root canals, due its broad antimicrobial spectrum [3-5] and its ability to promote organic tissue dissolution [6]. However, sodium hypochlorite is highly irritating when it contacts periapical tissues [7], reduces the resistance of teeth to fracture [8] and interferes negatively with bond strength of adhesive restorations to dentin [9,10]. Because of the adverse effects of this irrigant, researchers have developed alternative endodontic irrigants.

Calcium hypochlorite ($Ca(OCl)_2$) is a relatively stable chemical substance that is commonly used for industrial sterilization and water purification treatments [11]. According to previous studies, this substance shows antibacterial properties [12,13], even against *Enterococcus faecalis* [14]. In addition, this chemical auxiliary substance has ability to promote organic matter dissolution [15].

The dissolution of pulpal tissue can be improved by agitation and heat [16,17] and previous studies have suggested the use of passive ultrasonic irrigation (PUI) as a means to improve the action of endodontic irrigants [18,19]. The ultrasonic device promotes the creation of sonic waves in irrigating solutions, which could improve the ability of pulpal dissolution and help the cleaning of complex anatomic areas. However, there are no studies in the literature concerning the association of calcium hypochlorite with passive ultrasonic irrigation to evaluate its ability to dissolve pulpal tissue.

The aim of this study was to compare *in vitro* effectiveness of calcium hypochlorite and sodium hypochlorite associated with passive ultrasonic irrigation on pulpal tissue dissolution of bovine teeth.

METHODS

This study was approved by the Ethics Commission of the School of Dentistry of University of Passo Fundo (Passo Fundo, RS, Brazil).

Tissue preparation

Forty-five bovine incisors were extracted from animals killed for commercial purposes. The teeth were immersed in saline solution and stored at a temperature of -20ºC for a period not exceeding 72 hours to preserve their properties until required. The teeth were thawed at room temperature and two longitudinal grooves were prepared in buccal and lingual surfaces. Using a diamond disc (KG Sorensen, Barueri, Brazil) and running from the crown portion to the apex, the teeth were split in half. The pulp tissue was removed and washed with distilled water. Each pulp sample was divided into two pieces of similar volume, resulting in 90 pieces, each approximately 10-mm long and 2-mm thick with a standardized weight of 40±5 mg.

Preparation of solutions

The 2.5% and 5.25% NaOCl solution was prepared by external laboratory (Natupharma, Passo Fundo, RS, Brazil). A fresh $Ca(OCl)_2$ solution was made up from granules (R&D Laboratories Ltd, Antrim, Northern Ireland, UK) at the time of each experiment. The concentrations of 2.5% and 5.25% was prepared using distilled water (weight/volume [w/v] ratio) and mixed with a magnetic stirrer for 10 minutes. All irrigants were kept under refrigeration until required.

Classification of treatment groups

The 90 samples of bovine pulp tissue were randomly distributed into nine groups according to the dissolution procedure, as follows:

Group 1: distilled water (DW).
Group 2: 2.5% NaOCl
Group 3: 5.25% NaOCl
Group 4: 2.5% $Ca(OCl)_2$
Group 5: 5.25% $Ca(OCl)_2$
Group 6: 2.5% NaOCl + PUI
Group 7: 5.25% NaOCl + PUI
Group 8: 2.5% $Ca(OCl)_2$ + PUI
Group 9: 5.25% $Ca(OCl)_2$ + PUI

Each group was allocated 10 samples (n=10) that were distributed equally within the groups to match closely with the mean weight. Each sample of pulp tissue was weighed on a high precision balance (Sartorius BP61S, Göttingen, Germany), obtaining the measurement of initial weight (IW) in milligrams (mg).

In the groups 1 to 5, each sample was immersed in a plastic microtube (Axygen Inc., Union City, California) containing 2.0 mL of tested solutions for a period of 2 minutes. Then, after the tissue was removed carefully, the solution was discarded and the sample re-immersed in a fresh 2 mL aliquot of the test solution. This process was repeated at 2-minute intervals until the tissue dissolved completely—or up to 30 minutes.

In the groups 6 to 9, each sample was immersed in a plastic microtube (Axygen Inc., Union City, California) containing 2.0 mL of tested solutions for a period of 1.45 minutes. Then we performed passive ultrasonic irrigation (PUI), using the ultrasonic device (NAC Plus Ultrasonics, Adiel, Ribeirão Preto, SP, Brazil). The stainless-steel endodontic tip to a size ET40 (Satelec-Acteon, Mount Laurel, New Jersey) was inserted into the microtube containing the test solution and activated for 15 seconds; the pulp was immersed for a total of 2 minutes in the test solution. The scale power 2 for endodontics was used to promote the ultrasonic activation. Then, the tissue was removed carefully, the solution was discarded, and the sample re-immersed in a fresh 2 mL aliquot of the test solution. This process was repeated at 2-minute intervals until the tissue dissolved completely or up to 30 minutes.

After that, each sample of bovine pulp tissue was dried with absorbent paper and weighed once again on a high precision balance, obtaining the measurement of final weight (FW) in milligrams (mg).

Statistical Analysis

The percentage differences in weight for each sample after the dissolution process was calculated and determined the mean percentage loss. The percentage difference of mass loss between the initial and final weight represented the amount of pulp tissue dissolution for each procedure. The results were subjected to statistical analysis using two-way ANOVA and Games-Howel tests for post hoc comparisons at 5% significance level to verify if there were significant weight loss differences between the groups.

RESULTS

The mean and standard deviation percentages of weight reduction for the samples in the different tissue-dissolving solutions are expressed in Table 1. The two-way ANOVA showed significant differences among the groups, which depended on the use of ultrasound treatment (p<0.001). Group 1 (DW) was not able to promote pulpal tissue dissolution and was thus statistically different from all other groups (p<0.001). Groups 2 (2.5% NaOCl) and 4 (2.5% $Ca(OCl)_2$) showed lower mean percentages of weight reduction, when compared to Groups 3 (5.25% NaOCl) and 5 (5.25% $Ca(OCl)_2$), which showed statistical differences between them (p<0.001). The groups where $Ca(OCl)_2$ was used in the pulp tissue dissolution procedure (4 and 5) showed significant results when compared to groups where NaOCl was used (2 and 3). When analyzing the concentrations of 2.5% and 5.25% alone with no PUI, Groups 2 and 3 showed statistical differences between them (p<0.001). Group 5 (5.25% $Ca(OCl)_2$), 6 (2.5% NaOCl+PUI), 7 (5.25% NaOCl+PUI), 8 (2.5% $Ca(OCl)_2$+PUI) and 9 (5.25% $Ca(OCl)_2$+PUI) showed the highest mean percentage of weight reduction when compared to all other groups. However, there was no statistically significant difference between them (p<0.001).

Table 1. Mean ± standard deviation percentage of tissue dissolution for experimental groups

Groups	No PUI	PUI
NaOCl 2.5%	56.16±6.98^{D}	97.22±3.41^{A}
$Ca(OCl)_2$ 2.5%	76.88±6.64^{C}	98.21±5.55^{A}
NaOCl 5.25%	89.06±5.22^{B}	100.00±0.00^{A}
$Ca(OCl)_2$ 5.25%	96.91±3.82^{A}	100.00±0.00^{A}

NaOCl, sodium hypochlorite; $Ca(OCl)_2$, calcium hypochlorite;
PUI, passive ultrasonic irrigation.
Means followed by different letters have statistically significantly differences (p<0.05).

DISCUSSION

The elimination of pulp tissue is an important requirement for successful endodontic therapy, since the persistence of residual organic matter in the root canal system exerts a significant role in the progression of periapical pathologies [1]. To achieve this, an endodontic irrigant must have ability to promote dissolution of organic matter, to remove residual pulp tissue, infected dentin and bacteria, and contribute to adequate cleaning of root canal systems [20].

According to previous study, bovine muscle tissue was used in order to evaluate the effectiveness of sodium and calcium hypochlorite regarding to dissolution of organic matter [15]. However, this kind of tissue cannot be found in the root canal space of teeth and does not provide an adequate simulation of clinical condition. Bovine pulp tissue has shown morphologically and physiologically similar properties to human pulp tissue [21]. Moreover, previous studies have used the model of bovine pulp tissue to assess the dissolution ability of several endodontic irrigants [6,22,23]. For these reasons, this kind of tissue was used in the current study to test different concentrations of sodium hypochlorite and calcium hypochlorite in association with passive ultrasonic irrigation to promote pulp tissue dissolution.

Tissue dissolution is dependent on three factors: frequency of agitation, amount of organic matter in relation to the amount of irrigating and surface area of contact [16]. Okino et al. [6] have placed the fragments of pulp tissue in contact with 20 mL of each chemical auxiliary substance in a beaker, with no use of an agitation device. The current study associated PUI to promote agitation of irrigants and was standardized by using the same volume of irrigant (2mL) for the samples of the respective groups, besides the fact that all samples of bovine pulp tissue fragments showed similar mean weights (40±5 mg). In addition, the protocols of pulp dissolution were finished after 30 minutes and the test solution was changed at 2-minute intervals. This methodology attempted to simulate clinical practice and account for the maximum time that might be spent during the chemomechanical preparation of root canals.

However, using the current method, the solutions were placed in contact with pulp tissue without the presence of dentin. Dentin has a detrimental effect on the ability of NaOCl and $Ca(OH)_2$ to dissolve pulp tissue [24]. In addition, the manual or rotary instrumentation removes organic matter, increasing the contact surface of the remnant pulp tissue with the irrigant solution. Therefore, there are small pieces of pulp tissue and a large volume of irrigant solution, which increases the effectiveness of endodontic irrigants to promote the dissolution of pulp tissue.

Previous studies have reported results concerning time to total dissolution of pulp tissue [6] and percentage of pulp tissue dissolution [25] in order to compensate for the variability of fragment weight. This article reports the results as percentages of weight reduction, in accordance with the method of Dutta and Saunders [15]. The initial weight of all pulp tissue samples was standardized at 40 ± 5 mg, which eliminates the variability of fragment weight. Thus, we have a better way of representing the percentage difference of mass loss between the initial and final weight of pulp tissue dissolution of each procedure.

The results of current study demonstrated that Groups 2 (2.5% NaOCl) and 4 (2.5% $Ca(OCl)_2$) showed lower mean percentages of weight reduction, when compared to Groups 3 (5.25% NaOCl) and 5 (5.25% $Ca(OCl)_2$). These findings are in accordance with previous studies [17,26,27], depicting a concentration-dependent efficacy for hypochlorite solutions. NaOCl ionizes to liberate hypochlorous acid (HOCl) and hydroxyl ions in an aqueous environment. In the same way, $Ca(OCl)_2$ ionizes to liberate hypochlorous acid (2 HOCl) and calcium hydroxide ($Ca(OH)_2$). Saponification, amino acid neutralization and chloramination reactions contribute to tissue dissolution from hydroxyl ions and hypochlorous acid [28]. Thus, the increasing the concentration of hypochlorite solutions speeds up these reactions, enhancing their ability to promote pulp tissue dissolution.

Calcium hypochlorite ($Ca(OCl)_2$) is normally used for industrial sterilization and water purification treatments. This chemical substance has demonstrated its ability to promote neutralization of *Enterococcus faecalis* from the root canal system [14] and there is no consensus in the literature about its cytotoxicity. According to current study, the groups where $Ca(OCl)_2$ was used (4 and 5) showed better results for the pulp tissue dissolution procedure when compared to groups where NaOCl was used (2 and 3), and we analyzed the concentration of 2.5% and 5.25% alone with no PUI. $Ca(OCl)_2$ ionizes in an aqueous environment to liberate two molecules of HOCl and one molecule of ($Ca(OH)_2$). From this reaction, $Ca(OCl)_2$ liberates one more molecule of hypochlorous acid when compared to NaOCl, which ionizes only one molecule. Thus, the available chlorine is higher in $Ca(OCl)_2$ formulations, which helps explain its higher ability to promote pulp tissue dissolution. These findings are not in accordance with a previous study [15], where $Ca(OCl)_2$ and NaOCl showed similar ability to promote soft-tissue dissolution. This can be explained by noting the amount of time the tissue remained in contact with irrigant agents; it varied from 35 to 60 minutes, whereas the time of contact in our study did not exceed 30 minutes. Moreover, this chemical substance has demonstrated its ability to promote neutralization of *Enterococcus faecalis* from the root canal system [14], it can be easily obtained and there is no consensus in the literature about its cytotoxicity, bringing some beneficial properties into the root canal therapy.

The use of passive ultrasonic irrigation (PUI) as an auxiliary resource in endodontic therapy has been suggested as an alternative to increase cleaning and disinfection of the root canal system [18,29,30]. On the other hand, previous studies have shown that the use of passive ultrasonic activation associated with NaOCl and $Ca(OCl)_2$ did not improve the potential for decontamination in root canals infected with *Enterococcus faecalis* [14,19,31,32]. Our goals were to investigate the influence of passive ultrasonic irrigation over calcium hypochlorite and sodium hypochlorite regarding their effectiveness in promoting pulp tissue dissolution: Previous studies reporting the association between PUI and endodontic irrigants have been limited to endodontic decontamination procedures.

Previous studies have also reported that tissue dissolution is not affected by osmolarity or buffer capacity [33], but it is improved by agitation and heat [17,34]. These results are in accordance to our findings: Groups 6 (2.5% NaOCl+PUI), 7 (5.25% NaOCl+PUI), 8 (2.5% $Ca(OCl)_2$+PUI) and 9 (5.25% $Ca(OCl)_2$+PUI) showed the highest mean percentage of weight reduction when compared to all other groups. Although there was no statistically significant difference between these groups and Group 5 (5.25% $Ca(OCl)_2$), the groups in which PUI was performed obtained the highest percentage means of tissue dissolution for experimental groups. The proposed ability of ultrasonic devices to create sonic waves in irrigating solutions deposited inside the root canal might aid in the adherence of NaOCl and $Ca(OCl)_2$ to the residual pulp tissue, as well as increase the temperature of these endodontic irrigants. Based on the results of this study, the use of PUI can increase the potential of pulp tissue dissolution of NaOCl and $Ca(OCl)_2$ with regard to eliminating organic matter from the root canal space. In addition, passive ultrasonic activation of endodontic irrigants can help to remove microbial biofilm [35] and clean the surfaces of anatomical complexities in root canal systems [36].

CONCLUSION

The results of current study demonstrates that calcium hypochlorite along with passive ultrasonic irrigation, can aid in chemomechanical preparation of root canals, contributing significantly to dissolution of pulp tissue during root canal treatment. Further studies are necessary to analyze other desirable properties of calcium hypochlorite as well as any harmful effects for recognition of this substance as a viable alternative irrigant in endodontic therapy.

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