ORIGINAL RESEARCH

Comparative Evaluation of Human Pulp Tissue Dissolution by 500-ppm and 200-ppm Hypochlorous Acid and 5.25% Sodium Hypochlorite: An *In Vitro* Study

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Abstract

Aim: The aim of this study was to assess, *in vitro*, the human pulp dissolution capacity of 500 ppm and 200 ppm of hypochlorous acid in comparison with 5.2% sodium hypochlorite (NaOCl).

Materials and methods: Forty pulp tissue samples were standardized to a weight of 9 mg and divided into four groups according to the irrigating solution used: 5.25% NaOCl, 500 ppm hypochlorous acid, 200 ppm hypochlorous acid, and distilled water. Eppendorf tubes carrying 2 mL of the irrigants were taken and the pulp tissue samples were placed in the tubes for the specified time interval: Subgroup A: 30 minutes and subgroup B: 60 minutes. After the designated time interval, the solution from each sample tube was filtered using Whatman filter paper and left for drying overnight. The residual weight was calculated by filtration method. The mean dissolution time for each experimental group at the different time intervals was statistically analyzed.

Results: Mean tissue dissolution increases with an increase in the time period. Approximately 5.25% NaOCl was most effective at both time intervals followed by 500-ppm hypochlorous acid at 60 minutes. Least amount of tissue dissolution was shown by 200-ppm of hypochlorous acid at 30 minutes. Distilled water did not show the ability to dissolve human pulp tissue.

Conclusion: Within the limitations of the study, 5.25% NaOCl dissolved the pulp tissue most efficiently at both time intervals and both concentrations. Human pulp tissue dissolution by hypochlorous acid was found to gradually increase with time and with an increase in its concentration.

Clinical significance: With the basic information that hypochlorous acid does have the capacity to dissolve human pulp tissue, further research can be undertaken to assess methods to increase its efficiency. Sooner than later, hypochlorous acid may be able to completely replace the toxic NaOCl in clinical practice, as the irrigant of choice during root canal therapy.

Keywords: Hypochlorous acid, Irrigant, Pulp dissolution, Sodium hypochlorite.

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Introduction

The ultimate goal of endodontic treatment is to eliminate microorganisms present in the root canal system, remove pulp tissue that could serve as a substrate for microbial growth, and fill the endodontic space to prevent bacterial recolonization, so as to preclude apical periodontitis or allow its resolution.[1] Numerous studies have shown that currently used biomechanical and chemo-mechanical methods of root canal preparation do not completely cleanse the root canal system.[2] Irrigation plays an indispensable role in endodontic treatment as it facilitates the removal of microorganisms, tissue remnants, and dentin chips from the anatomically complex root canal through a flushing mechanism. Certain irrigating solutions dissolve either organic or inorganic tissue in the root canal and, in addition, have antimicrobial activity, actively killing bacteria and yeasts.[3]

Sodium hypochlorite (NaOCl) is still the most effective "gold standard" irrigant. Its advantages are twofold: pulpal dissolution and antimicrobial effect; however, there are significant biological toxicity risks if NaOCl is expressed into the periodontal ligament space with the outcomes being significantly worse at higher concentrations.[4]

The need remains for an irrigating solution having the same advantages as NaOCl while overcoming its disadvantages of storage risks and toxicity caused by inadvertent extrusion.[5]

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Hypochlorous acid (HOCl), discovered in 1834 by French chemist Antoine Jerome Balard, is an infection-fighting, endogenous compound produced in all mammals by neutrophils, eosinophils, and lymphocytes. It is a powerful oxidizing agent that dissociates into H^+ and OCl^- in an aqueous solution, denaturing and aggregating proteins. Mildly acidic HOCl solutions show greater antimicrobial potential than NaOCl solutions within their effective antimicrobial concentration range.[6]

Hypochlorous acid has been successfully used in the field of medicine for wound debridement and wound bed preparation.

A study conducted by Hiebert and Robson[7] concluded that compared with cytotoxic wound irrigants, such as chlorhexidine and Dakin's solution, HOCl is a nontoxic alternative. Ultrasonic debridement of chronic wounds with HOCl drastically lowered the tissue bacterial count.

Most of the previous studies in dentistry have focused on the antibacterial and smear layer removal properties of HOCl.[5,8] Studies have also been performed to compare the pulp dissolution capacity of other chlorine derivatives such as chlorine dioxide and calcium chloride,[9] but there is no literature available on HOCl.

Hence, this *in vitro* study aimed to compare human pulp tissue dissolution by 500 ppm and 200 ppm of HOCl and 5.25% NaOCl at two different time intervals of 30 minutes and 60 minutes.

Materials and Methods

This *in vitro* study was conducted in the district of Davanagere, Karnataka, over a period of 3 months from October to December 2022. Sixty vital, intact, premolar teeth, freshly extracted for orthodontic purposes, were collected from the Department of Oral and Maxillofacial Surgery. Fractured or carious teeth were discarded.

This study followed a methodology similar to that used by Taneja et al.[9] Using a round bur, two longitudinal grooves were made on the proximal surfaces of the teeth. A chisel and mallet were used to split the teeth into two halves. The entire pulp tissue was removed, cut with a no. 15 BP blade, and placed on a pre-weighed Whatman filter paper to obtain 9.0 mg of tissue that was considered as the baseline, initial weight reading. Out of the 60 teeth, 40 pulp tissue samples having a standardized weight of 9.0 mg per sample were obtained and divided into four groups as shown in Flowchart 1.

In group I: 10 Eppendorf tubes were filled with a measured volume (2 mL) of 500 ppm HOCl. In group II: 10 Eppendorf tubes were filled with a measured volume (2 mL) of 200 ppm HOCl. In group III: 10 Eppendorf tubes were filled with a measured volume (2 mL) of 5.25% NaOCl. In group IV: 10 Eppendorf tubes were filled with a measured volume (2 mL) of distilled water.

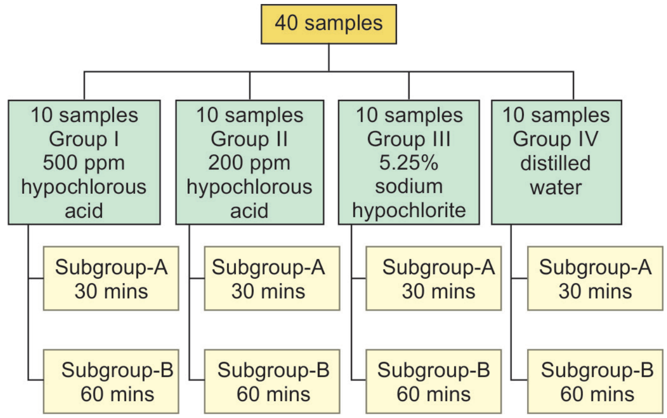
Commercially available 500-ppm HOCl (EKO Power HOCl; Ecocre^{TM}) and 5.25% NaOCl (NICE^{R}) were used. A solution of 200-ppm HOCl was prepared by diluting 100 mL of 500-ppm HOCl with 150-mL distilled water.

Each group was further divided into two subgroups according to the different time periods for which the samples were immersed in their respective irrigant solutions. Subgroup A: 30 minutes and subgroup B: 60 minutes, each having five Eppendorf tubes (Flowchart 1).

Pulp tissue was placed in the Eppendorf tubes carrying the respective irrigants and incubated at 37°C. Once the specified time had passed, the solutions from each tube were filtered through a Whatman filter paper. The filter papers were subsequently dried overnight and measured on an analytical balance. The weight of the dissolved pulp tissue was obtained by subtracting the weight of the dried filter paper (with residue) from the weight of the initial filter paper (before filtration). Thus, by filtration method, the quantity of human pulp tissue dissolved by the various irrigating solutions at different time intervals was quantitatively measured.

The mean dissolution time for pulp tissue by different groups at different time intervals was statistically analyzed by analysis of variance (ANOVA), and *post hoc* analysis [Tukey's honestly significant difference (HSD) test] was carried out for intergroup comparison by using the Statistical Package for the Social Sciences (SPSS) software program, version 22.0.

Flowchart 1: Distribution of the samples



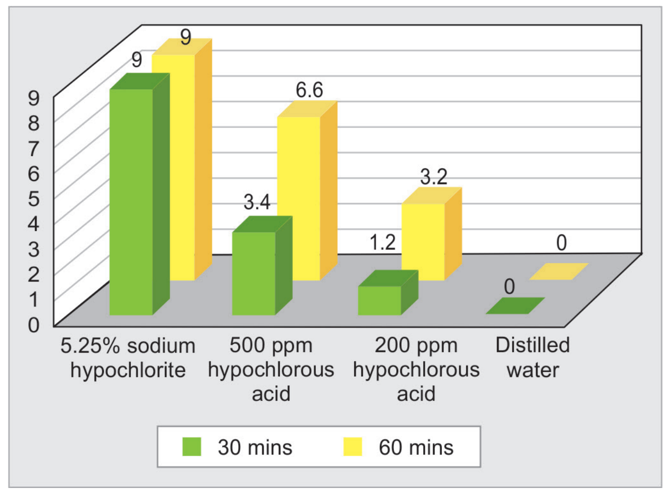


Fig. 1: Mean pulp tissue dissolution by various experimental solutions

Results

Results of this study showed that only 5.25% NaOCl was able to completely dissolve human pulp tissue as compared with the other groups. Complete dissolution of the pulp tissue was noted at the end of 30 minutes.

Around 500 ppm HOCl was able to dissolve a mean pulp tissue weight of 3.4 mg in 30 minutes and 6.6 mg in 60 minutes, which indicates that the pulp tissue dissolution capacity of HOCl increases over time. 200-ppm HOCl showed lower pulp tissue dissolution capacity than 500 ppm with a mean tissue dissolution weight of 1.2 mg at 30 minutes and 3.2 mg at 60 minutes (Fig. 1).

Distilled water showed no pulp tissue dissolution ability even at the end of 60 minutes.

Hence, according to the results of this study, the mean dissolution time for pulp tissue was found to increase over time for all the groups except the distilled water group. Distilled water showed no dissolution of the pulp. A statistically significant difference was found between all the groups (Table 1).

Discussion

In this study, HOCl was chosen as an experimental irrigant and compared with NaOCl as it shows much lesser toxicity and is far less irritating when applied to the human body as compared with NaOCl.

Table 1: Mean pulp tissue dissolution time by various groups

Time	Test group	N	Mean (mg)	Standard deviation
Subgroup A (30 min)	5.25% sodium hypochlorite	5	9.0000	0.00000
	500-ppm hypochlorous acid	5	3.4000	0.54772
	200-ppm hypochlorous acid	5	1.2000	0.44721
	Distilled water	5	0.0000	0.00000
Subgroup B (60 min)	5.25% sodium hypochlorite	5	9.0000	0.00000
	500-ppm hypochlorous acid	5	6.6000	0.54772
	200-ppm hypochlorous acid	5	3.2000	0.44721
	Distilled water	5	0.0000	0.00000

When in contact with organic matter, it does not form carcinogenic chlorinated hydrocarbons.[10]

In the medical field, it has been used as an irrigant for wound debridement to accelerate endogenous healing. Hiebert and Robson,[7] in their study, concluded that HOCl irrigation with ultrasound debridement causes a reduction of bacterial growth in chronic open wounds more efficiently than saline alone. According to Robson et al.,[11] the important factors for efficient wound bed preparation are debridement of necrotic tissue and debris, reduction in excessive wound exudate, reduction in the tissue bacterial level, removal of deleterious chemical mediators, and acceleration of endogenous healing, all of which are desired actions of an "ideal root canal irrigant."[12] Currently, there is no literature available evaluating the tissue-dissolving capacity of HOCl. In this study, 500 ppm and 200 ppm of HOCl have been compared as these are the recommended and commonly used concentrations of HOCl as a disinfectant.[6]

Hypochlorous acid has a broad spectrum of antimicrobial activity and does not show cytotoxic activity. In a study by Wang et al.,[13] HOCl was fully capable of inactivating all groups of gram-negative and gram-positive bacteria, yeast, and fungi, including *Staphylococcus aureus*, methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus faecium*, and *Bacillis anthracis* spores. It was also shown to be nonirritating and nonsensitizing in animal models.

Considering the recent outbreak of coronavirus disease-2019 (COVID-19) virus, HOCl is listed in "List N: Disinfectants for use against SARS-CoV-2" by the US Environmental Protection Agency.[14] In a review study done by Drozdzik et al.,[15] evaluating the existing evidence related to the molecular processes of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in the oral cavity, it was found that pulp tissues have a tendency to be infected by SARS-CoV-2. Hence considering its efficacy in destroying the COVID-19 virus, HOCl has the potential to be called an "ideal endodontic irrigant."

Results of this study show that 5.25% NaOCl most effectively dissolved human pulp tissue at both time intervals. This is due to the liberation of hydroxyl ions (OH) and HOCl when NaOCl ionizes in an aqueous environment. When the level of OH decreases due to its consumption during the amino acid neutralization and saponification reactions, the pH also decreases, thereby favoring the formation of HOCl molecules. This initiates the chloramination reaction that results in the hydrolysis and degradation of amino acids.[16] The result is in agreement with a study conducted by Hand et al.,[17] to determine the effect of dilution on the ability of NaOCl to dissolve necrotic tissue. The authors compared 5.25% NaOCl, 2.5% NaOCl, 1.0% NaOCl, 0.5% NaOCl, normal saline solution, distilled water, and 3% hydrogen peroxide. Necrotic tissue specimens were placed in contact with various test solutions and the resulting change in tissue weight was recorded using the filtration method. The study concluded that 5.25% NaOCl was the most effective tissue solvent.

In comparison with NaOCl, the mean tissue dissolution by HOCl was significantly less at both concentrations and at both time intervals. This might be due to the low pH of HOCl (pH = 3.5–5) as compared with the high pH (pH = 12) of NaOCl that favors tissue dissolution.[13] Studies have suggested that higher pH levels resulted in greater tissue dissolution and lower the pH, more time was needed for solution contact for tissue dissolution.[2] Hence, HOCl was less effective in dissolving pulp tissue at 30 minutes than at 60 minutes.

Although no studies have been done to evaluate the human pulp tissue dissolution capacity of HOCl, the results of this study are similar to those obtained by Taneja et al.,[9] where the human pulp tissue dissolution capacity of chlorine dioxide, calcium hypochlorite, and NaOCl have been compared. Chlorine dioxide and calcium hypochlorite showed less tissue dissolving properties than NaOCl.

Results of this study showed no dissolution of human pulp tissue by distilled water at either time intervals. This is in agreement with an *in vitro* study by Gordon et al.,[18] who determined the solvent effect of various dilutions of NaOCl solution and distilled water on vital and necrotic tissue. The study concluded that distilled water was ineffective as a solvent of the vital tooth pulp.

The authors of this *in vitro* study used commercially available and commonly used disinfectant preparations of HOCl. The efficiency of this study may increase with the use of freshly prepared, highly potent solutions of HOCl.

As this is a first-of-its-kind study, more research can be conducted on this novel irrigant and methods to increase the efficiency of HOCl as a tissue solvent by increasing its pH or by the use of ultrasonics or lasers could be tested.

Conclusion

Within the limitations of this *in vitro* study, 5.25% NaOCl was found to be the most effective irrigant to dissolve pulp tissue. Although HOCl did not dissolve pulp tissue as effectively as NaOCl, this study proves that it does have the potential to be a tissue solvent. The human pulp tissue dissolution capacity of HOCl was more effective at a higher concentration and at a longer tissue contact time duration. Distilled water showed no tissue dissolution capabilities.

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