Original Article

Effect of different chelating agents on bovine tissue dissolving capacity of sodium hypochlorite

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ABSTRACT

Background: The success of endodontic therapy depends on thorough cleaning of the canal system. This is done by the use of various irrigants together with mechanical cleaning. The most popular irrigant used is sodium hypochlorite (NaOCl) in different concentrations. It has an added advantage in that it is a tissue dissolvent. To improve the removal of the smear layer in the canal, NaOCl is used with chelating agents such as ethylenediaminetetraacetic acid (EDTA), etidronic acid, or chitosan. The aim of this study was to evaluate the effect of different chelating agents when used with NaOCl on tissue dissolving capability.

Materials and Methods: Cleaned bovine tissue was cut into equal pieces of 4 mm × 4 mm × 2 mm and divided into 60 samples having a weight of 70 ± 3 mg. Irrigant solutions were divided into four groups. Group 1: 3% NaOCl, Gp 2: 3%NaOCl + 17% EDTA, Group 3: 3% NaOCl + 18% Etidronic acid, and Group 4: 3% NaOCl + 0.2% chitosan in acetic acid. Samples were taken into test tubes and kept in 10 ml solution of each group for 10 min. Later, samples were taken out from solution and washed with distilled water, blotted dry, and weighed again for change of weight. The difference between the initial and final weights was used to calculate the weight loss.

Results: There was a significant difference between the sample weight before and after treatment for all the groups. NaOCl (Group 1) showed best percentage difference of weight loss, followed by NaOCl + Etidronic acid ($P < 0.05$), then NaOCl + EDTA ($P < 0.05$) and least with NaOCl + Chitosan ($P < 0.05$).

Conclusion: Etidronic acid has shown better dissolution capacity as compared to EDTA and chitosan group when used with NaOCl.

Keywords: Chitosan, ethylenediaminetetraacetic acid, etidronic acid, sodium hypochlorite

INTRODUCTION

Successful endodontic therapy depends on the triad of effective instrumentation, adequate irrigation, and three-dimensional obturation of the entire root canal system.[1] During and after instrumentation of the root canal, the irrigating solution helps in removal of microorganisms, tissue remnants, and dentinal chips from the root canal system by dissolution and flushing mechanism. Irrigants can dissolve either organic or inorganic tissue in the root canal system. In addition, several irrigating solutions have antimicrobial activity on direct contact.[1]

Sodium hypochlorite (NaOCl) has many desired properties of an ideal irrigating solution such as broad antimicrobial activity, efficacy against organized biofilms, tissue dissolving ability, and prevent the formation of smear layer while instrumenting the canal.[1] The concentrations used ranges between 0.5% and 6%. Due to its antimicrobial property, most of bacteria are destroyed immediately while in direct contact. A concentration of 5.25% for 40 min contact time is found to be most effective in this regard.[1] It dissolves the organic

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components of dentin, i.e., remnants of pulp and collagen, a major advantage.[2]

Ethylenediaminetetraacetic acid (EDTA) is a chelating agent.[3] EDTA reacts with the calcium ions in dentin and forms soluble calcium chelates. EDTA can decalcify dentin to a depth of 20–30 μm in 5 min.[4] A continuous rinse with 5 ml of 17% EDTA for 3 min can efficiently remove the smear layer from the walls of the canal.[5]

An alternative chelating agent to EDTA or citric acid is 1-hydroxyethylidene-1, 1-bisphosphonate (HEBP), also named as etidronic acid or etidronate.[4] It is commonly used in a concentration of 20%. It has applications in the pharmaceutical industry as an osteoporotic agent, in the metal industry as an anticorrosive agent and in the soap industry to prevent oxidation and rancidification of fatty acids.[4]

Chitosan is a natural polysaccharide, a nontoxic cationic biopolymer usually obtained by alkaline deacetylation from chitin. It presents with biocompatibility, chelating capacity, and antimicrobial effects against a broad range of Gram-positive and Gram-negative bacteria as well as fungi.[6-9]

It has been shown that mixing of EDTA with NaOCl during chemo-mechanical procedure can reduce the available free chlorine,[10] thus affecting the dissolution and antibacterial ability of NaOCl. Therefore, a chelating agent that does not affect the tissue dissolving ability of NaOCl is required. The purpose of this study is to evaluate the effect of EDTA, etidronic acid, and chitosan on bovine tissue dissolving capacity of NaOCl.

MATERIALS AND METHODS

Four irrigant solutions were used in this study. The materials chosen were Group I-3% NaOCl (Parcan, Septodont India, Maharashtra), Group II-3% NaOCl + 17% EDTA (Smearclear, Sybron Endo, CA), Group III-3% NaOCl + 18% Etidronic acid (Sigma Aldrich, USA), and Group IV-3% NaOCl ± 0.2% Chitosan in acetic acid (Aura chemicals, Chennai).

Sample preparation

Cleaned bovine tissue procured from abattoir was used as a tissue sample in the experiment. Tissue was kept frozen at −15°C and 100% humidity. Frozen tissue was cut into equal pieces of 4 mm × 4 mm × 2 mm using stainless steel surgical blade into 60 samples. Samples were blotted dry and weighed using analytical balance. Samples had a weight of approximately 70 mg using precision scale with a difference of ±3 mg between samples. Care was taken to prepare sample size (4 mm × 4 mm × 2 mm) and sample weight 70 ± 3 mg as similar as possible. Experiment was conducted in closed chamber to prevent any disturbances in the weighing procedure. No agitation in any of the solutions was carried out.

Dissolution test

Procedure was done at room temperature which was 31°C on the day. The temperature was measured with the help of glass thermometer. The preweighed bovine samples were taken into test tubes and grouped randomly. These samples were kept in 10 ml solution of each group for 10 min. Later, samples were taken out from respective solutions and washed with distilled water, blotted dry, and weighed again for loss of weight.

The difference between the initial and final weights was used to calculate the weight loss, thereby representing the dissolving ability of each substance/mixture. The results were subjected to *post hoc t*-test for analysis.

RESULTS

The mean values of pulpal tissue weights for each group (preexposure and postexposure) were calculated. The mean and standard deviation values for the initial and final pulp tissue weights before and after the dissolving test (preexposure and postexposure) are shown in Table 1. (SPSS) Statistical Package for the Social Sciences (IBM , USA) 21.0 software was selected for analysis after consideration of the number of groups and the intergroup comparison required.

There was a statistically significant difference between the sample weight before and after treatment for all the groups ($P < 0.05$). NaOCl showed best percentage difference of weight loss, followed by NaOCl + HEBP that was followed by NaOCl + EDTA, and least with NaOCl + Chitosan. When solubility was compared between the groups, the difference between NaOCl + EDTA and NaOCl + Chitosan group was not statistically significant ($P > 0.05$). Tissue dissolution capacity of NaOCl + HEBP was more as compared to NaOCl + EDTA and NaOCl + Chitosan group. The difference was

Table 1: The mean and standard deviation values for the pulp tissue weights before and after the dissolving test

Solution	Pre exposure	Post exposure	% difference
NaOCl	70.1 (±1.55)	47.02 (±3.44)	32.91
NaOCl+ 17% EDTA	70.7 (±1.77)	60.71(±1.56)	14.09
NaOCl+ 18% HEBP	69.7(±1.66)	55.02(±2.67)	21.06
NaOCl+ 0.2% Chitosan	70.76(±1.84)	62.46(±3.79)	11.74

NaOCl; Sodium hypochlorite, HEBP; 1-hydroxyethylidene-1, 1-bisphoshonate, EDTA; Ethylene diamino tetraacetic acid

statistically significant. Intergroup variability has been shown through *post hoc t*-test [Table 2].

DISCUSSION

There have been experimental models which have been tested to determine the dissolving effect of NaOCl.[11-14] These methods assessed the weight loss of the sample, the time required for dissolution, or provided data for microscopic examination and a visual assessment of the size of the remaining tissue. The time required for sample dissolution provides results that can be quantified and allow a more reliable analysis.

NaOCl has been systematically used as an endodontic irrigant for the chemomechanical preparation of root canals as it is having excellent antimicrobial action, has capacity to dissolve organic tissue remnants and it improves the action of instruments by lubricating the root canal walls.[15] Although it is known that NaOCl can affect organic tissues, little research has been done about its dissolving capacity when it is combined with other auxiliary irrigation solutions.[16]

This study used an *in vitro* protocol that allowed comparison of the tissue dissolving ability of NaOCl alone and in combination with different chelating agents such as etidronic acid, EDTA, and chitosan. It has been seen that HEBP and chitosan have chelating properties similar to EDTA with the additional advantage of antimicrobial properties.[9]

Tissues from a number of different sources have been used in previous studies related to the tissue-dissolving ability of NaOCl.[17] Porcine muscle tissue, rabbit liver, rat connective tissue, pig palatal mucosa, bovine muscle tissue, and bovine pulp have been used in various studies to determine tissue-dissolution capacity of different irrigating solutions.[18] The reasons for using different tissues instead of the dental pulp is the availability and easier standardization of the surface area of each specimen.[19]

The use of a combination of NaOCl and EDTA is effective in the removal of organic and inorganic debris from the root canal system. EDTA is a Ca^{+2} chelating agent and therefore it is capable of removing the smear layer. EDTA may be used as a final flush to open up dentinal tubules, thus allowing a greater number of lateral canals to be filled or may also be used alternately with NaOCl during the whole preparation of the root canal.[20]

Etidronic acid (also known as or HEBP) is a biocompatible chelating agent that can be used in combination with NaOCl and has adequate calcium chelating capacity.[21,22] Etidronic acid is a soft chelating agent and a potential alternative to EDTA and can be used to remove the inorganic debris such as smear layer from the root canal which is produced during instrumentation of the root canal.[23]

Chitosan and etidronic acid when used at concentrations of 0.2% and 18%, respectively, have been shown to result in good smear layer removal.[24] Neither of these agents possess adequate tissue dissolving action when used independently but mixing with 5.25% NaOCl has been shown to impart this property.[1] There are no studies to date comparing the bacterial inhibitory action of chitosan and etidronate head-to-head, although both chemicals are believed to have some effect.[25]

In addition, no previous studies are available for interaction between NaOCl and chitosan on their tissue dissolving capacity. In 2005 Zehnder *et al*. evaluated the effect of chelators on loss of available chlorine in NaOCl and found that EDTA caused immediate loss of chlorine from irrigation solution, while HEBP did not.[26] This could be the reason for reduced tissue dissolution when NaOCl was combined with EDTA as compared to HEBP.

Similar results were obtained by Arias-Moliz *et al*. in 2014 where they concluded that HEBP did not interfere with the ability of NaOCl to kill E fecal is grown in biofilms and inside dentinal tubules.[27]

Table 2: *Post hoc t*-test showing statistical difference values among the groups

Group	1	2	3	4
1	<0.05*	<0.05*	<0.05*	<0.05*
2	<0.05*	<0.05*	<0.05*	0.377
3	<0.05*	<0.05*	<0.05*	<0.05*
4	<0.05*	0.377	<0.05*	<0.05*

CONCLUSION

According to results obtained, NaOCl had the greatest ability to dissolve bovine tissues. This tissue dissolution action is promoted by sodium hydroxide, one of the by-products of NaOCl, which is a powerful organic and fat solvent. HEBP has shown better dissolution capacity as compared to EDTA and chitosan group. Chitosan group has shown minimal dissolution capacity when used in combination with NaOCl.

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Conflicts of interest
There are no conflicts of interest.

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