Antibacterial Effect of Sodium Hypochlorite Gel and Solution on Enterococcus faecalis

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Abstract

Introduction:
The purpose of this study was to compare the antibacterial effect of sodium hypochlorite gel and solution on Enterococcus faecalis (E. faecalis). The main purpose of a root canal treatment is to eliminate the bacteria and their products. Sodium hypochlorite solution has excellent antibacterial properties, but also some negative features.

Materials and methods:
Fifty six single root straight canals were instrumented with Ni-Ti rotary files (ProTaper S1,S2,F1,F2,F3) and contaminated with E. faecalis. Then they were divided into four groups. Group A used sodium hypochlorite (NaOCl) 5.25% solution, Group B used NaOCl 5.25% gel, Group C used normal saline solution, and Group D used no irrigation. Microbiological samples were collected with sterile paper points. Statistical analysis was performed using MannWhitney U-test. The significance level was set to $p<0.05$.

Results:
In Groups A and B, no sign of E. faecalis presence was observed. Groups C and D showed presence of E. faecalis in all samples.

Conclusion:
NaOCl 5.25% solution and gel showed the same effectiveness. Therefore, NaOCl 5.25% in the form of a gel can be recommended as a safe and controllable intracanal irrigant.

Key words:
•Enterococcus faecalis •Endodontics •Sodium Hypochlorite

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Introduction

One of the most important causes of failure in endodontic treatment is improper elimination of bacteria from root canal systems.(1) For this reason, elimination of bacteria and their products is the main goal of endodontics.(1-2) Enterococcus faecalis (E. faecalis) is one of

the most resistant microorganisms in the root canal system[3] and is the most commonly-identified species in post treatment diseases.[4] E. faecalis is associated with persistent endodontic diseases because it is able to penetrate into dentinal tubules, tolerate food deprivation, and is able to produce biofilms.[2]

Chemical irrigation is used to disinfect root canal system, complementary to mechanical instrumentation. One of the most potent and commonly-used root canal irrigants is sodium hypochlorite (NaOCl).[5-6] NaOCl solutions are commonly used, economical, accessible, and have a good shelf life.[7] However, in case of apical extrusion or leakage through rubber dam isolation, NaOCl is cytotoxic and can induce allergic reactions.[8] Moreover, it has unpleasant taste and smell. Application of NaOCl as gel provides better control and exhibits reduction in apical extrusion and overall side-effects of NaOCl.[5,9] Therefore, the purpose of this study was to compare the antibacterial efficacy of NaOCl solution and gel on E. faecalis within root canals.

Materials and Methods

Fifty six non-carious, unrestored, matured single-rooted teeth with straight canals were used in this in vitro study. In addition, the teeth exhibited no resorption, cracks or fractures, calcifications, and anomalies. All the teeth were cleaned to remove any superficial debris, and were stored in 3% chloramine-T solution. The crowns were then removed with a high speed hand piece and a diamond bur to standardize the remaining root length to 13 mm. The working length was determined with a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) 1 mm shorter than the apical foramen. Then, rotary instrumentation (ProTaper S1,S2,F1,F2,F3, Dentsply Maillefer, Ballaigues, Switzerland) was carried out using single-length technique. Each tooth was irrigated with 5 mL of distilled water and sterilized in an autoclave at temperature of 121 °C for 20 minutes. Enterococcus faecalis (ATCC29212) (Pastor, Iran) was prepared in Brain Heart Infusion (BHI) broth (Pronadisa, Spain) in the bacteriology laboratory of Guilan University of Medical Sciences. The optical density was 1.5×108 CFU/mL and was standardized to McFarland 0.5 BaSO4. The teeth were incubated for 48 hours in BHI broth at 37 °C.

The teeth were randomly divided into four groups (n = 14): Group A (solution); Group B (Gel); Group C (normal saline); and Group D (positive and negative control). Ten µL of E. faecalis suspension was carried out to each root canal (except for the negative control group which was not contaminated) and was incubated for 24 hours in 37 °C.

Group A root canals were irrigated with 1 mL NaOCl 5.25% solution (Cerkamed, USA) using a 30 gauge syringe needle 1 mm shorter than working length. Then a number 15 K-file was carried to the working length to assure the complete penetration of NaOCl solution. After 1 minute the root canals were irrigated with 1 mL normal saline solution.

Group B root canals were filled with 1 mL NaOCl 5.25% gel (Unilever, Iran) and a number 15 K-file was carried to the working length to assure the complete penetration of NaOCl gel. After 1 minute the root canals were irrigated with 1 mL normal saline solution.

Group C root canals were irrigated with 1 mL normal saline solution. Then, a number 15 K-file was carried to the working length to assure complete penetration. After 1 minute the root canals were irrigated with 1 mL normal saline solution.

Group D root canals which were sterilized as negative control (n = 7) or were contaminated with E. faecalis as positive control (n = 7) were not irrigated with any solution or gel.

Then, sterile paper points (ProTaper F3 Paperpoint, Dentsply Maillefer, Ballaigues, Switzerland) were introduced to the working length of each root canal and remained for 1 minute to collect samples. Then, the paper points were transferred into a tube containing 5 mL BHI broth and vortexed for 5 minutes. All the procedures were carried out in a laminar flow chamber with sterile instruments. After incubation at 37 °C for four days, each tube was checked for the presence of turbidity and transferred to blood agar culture plates to check the presence of E. faecalis. Moreover, Gram staining was done to confirm the presence of E. faecalis.

Statistical analysis was performed using Mann Whitney U-test in SPSS21 software. The significance level was set to $p<0.05$.

Results

E. faecalis was not detected in Groups A and B. Moreover, in the negative control group E. faecalis was not present. Growth of E. faecalis was detected in all samples of Group C and the positive control group. Mann Whitney U-test showed a significant difference between NaOCl irrigation and normal saline irrigation. Also there is a significant difference between NaOCl gel and normal saline irrigation ($p < 0.0001$). but no significant difference between Positive control (D) and normal saline irrigation ($p = 0.998$). (Table 1)

Table 1. Bacterial presence in each group (distribution percentage is written in parentheses)

Groups	Solution (A)	Gel (B)	Normal Saline (C)	Positive Control (D)
Bacterial presence	0(0%)	0(0%)	14(100%)	14(100%)

Discussion

E. faecalis is one of the most resistant microorganisms associated with post treatment diseases.[4] Mechanical instrumentation and the physical flow of irrigating solution can reduce the bacterial load. However, it was reported that normal saline irrigation combined with mechanical instrumentation did not show any significant reduction of E. faecalis.[6,10] Consistent with the aforementioned studies, all samples of our study which were irrigated with normal saline (Group C) showed complete bacterial growth.

An ideal intracanal irrigant is required to kill microorganisms, dissolve necrotic tissues, and does not irritate healthy tissues.[11] NaOCl has been in use for almost a century.[7] It is a potent antibacterial agent against E. faecalis, depends on concentration and time of exposure.[12] NaOCl solution is the most commonly used root canal irrigant, however its toxic and irritative effects on vital tissues and the risk of hypochlorite accident in case of apical extrusion are its shortcomings. In this study, we compared the gel form of NaOCl with NaOCl solution. Gel form of NaOCl was selected with the purpose of reducing the risk of apical extrusion and enhancing manipulation, control and overall side-effects of the solution form.[5,9] The results of the present study demonstrated no significant difference between antibacterial efficacy of 2.25% gel and 2.25% solution and both were highly potent, which was consistent with the results of the study of Al-sudani et al.[9] In a study conducted by Zand et al., the gel form of NaOCl 2.5% exhibited significantly lower antimicrobial efficacy in comparison with 2.5% and 5.25% solutions.[5] It may be related to high viscosity of the gel form which is less able to penetrate into canal irregularities and the depth of dentinal tubules. We used NaOCl in concentrations of 5.25% to achieve the highest potential of both gel form and solution.

This study has some limitations of using only one chemical irrigant and not considering the biofilm formation ability of E. faecalis. Although NaOCl was very potent in our study, we did not consider deeply residing bacteria in biofilms and dentinal tubules which may survive after treatment. In addition, more studies need to be done to introduce an ideal irrigant.

Conclusion

5.25% NaOCl gel exhibits the same effectiveness as 5.25% NaOCl solution, and it can be recommended as an effective intracanal irrigation agent.

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