An In-vitro Evaluation of the Ability of 5.25% NaOCl in the Elimination of Enterococcus Faecalis from Root Canal

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Statement of Problem: Sodium hypochlorite (NaOCl) have been widely used as an irrigant since it has been introduced to endodontics by walker in 1936, because of its bleaching, deodorizing and tissue dissolving properties. It should be used clinically in concentrations of 3% to 5%.
Purpose: The aim of this study was to evaluate the effectiveness of the NaOCl to eliminate Enterococcus faecalis (E.f) from root canals in comparison with Normal saline.
Materials and Methods: In an interventional study forty freshly extracted single canal human teeth were chosen. They were sectioned at the CEJ, instrumented and Sterilized. Then they were contaminated with E.f solution and incubated. These samples divided into two groups randomly. Root canals were irrigated and filled with 5.25% NaOCl for five minutes in group one, and with normal saline in group two. Then samples were obtained from canals with sterile paper points and cultured for four days. The appearance of turbidity in cultured solutions was the indication to of E.F presence. In order to confirm the specific presence of E.F, three complementary microbiologic tests were applied.
Results: All cultures obtained from NaOCl group were negative and all of normal saline group were positive.
Conclusion: these results indicate the ability of 5.25% NaOCl to eliminate E.F in prepared root canals with wide diameter.

Key words: Root canal irrigation; Sodium hypochlorite (NaOCl); Normal saline; Enterococcus faecalis (E.f)

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It has been demonstrated that bacteria and their products play an essential role in the development and perpetuation of pulpal and periapical diseases.(1,2)

Although the root canal flora is dominated by obligate anaerobic bacteria, some facultative strains, e.g Enterococcus Faecalis (E.f), have been involved in persistent infections, influencing the prognosis of the root canal treatment.(3)

Once bacteria are established in the root canal system, they cannot easily be reached by the host defense system.

Hence, infections of endodontic origin are treated mainly by means of mechanical procedures aided by chemical substances.

Numerous irrigants have been recommended for use in the treatment of root canal infections. Sodium hypochlorite (NaOCl) have been widely used as an irrigant since it has been introduced to endodontics by walker in 1936, because of its bleaching, deodorizing and tissue dissolving properties.[4]

In order to take advantage of NaOCl ability to destroy all microorganisms upon direct contact and its unique ability to dissolve pulp tissue from all aspects of the root canal system, It should be used clinically in concentrations of 3% to 5%.[5,6].

The potential for an irrigant is maximized when it is heated, flooded into shaped canals, and given ample time to work.[7,8,5]

Based on premises, the purpose of this study was to evaluate effectiveness of 5.25% NaOCl in eliminating E.f from shaped and enlarged root canals in a short time.

Materials and Methods

In an interventional study forty freshly extracted human teeth with single canal were selected. The crowns were removed by sectioning the teeth at the CEJ.

The root canals were instrumented 1mm beyond the apical foramen with k-type files up to size 45 and then flared to size 60. Irrigation with tap water was performed during the enlarging procedures. The apical foramen was sealed by means of epoxy resin to prevent bacterial leakage. To make both handling and identification easier, the teeth were then mounted vertically in plaster blocks and sterilized in an autoclave for 20 minutes at 121ºC and 15 PSI pressure.

Pure culture of E.f (ATCC 29212), grown in brain infusion broth- BHI- (Merck, 10493, Germany) was used. A Suspension of E.f cells was prepared in BHI broth, and by comparing its turbidity to a McFarland 0.5 $BaSO_4$ standard it had an optical density of approximately 1.5? 10^8 colony forming units /ml ($^{CFU}/_{ml}$).

In a Laminar air flow cabinet; each root canal was inoculated with 10 µL of the E.f suspension using sterile 1ml Insulin syringes. The blocks were then placed inside stainless steel boxes and incubated at 37ºC for 24h.

After incubation, the contaminated root canals were irrigated with 5 ml sterile saline and dried with resterilized paper points. Then they were randomly divided into two groups.

In group one, 20 root canals were irrigated with 5 ml of a 5.25% NaOCl solution (Bojneh -Iran). It was agitated by hand file size 25 and left in the root canal for 5 minutes. Group two was treated similar to group one but irrigation solution was normal saline (Samen- Iran).

Irrigants were delivered in the canals by means of a 5-ml syringe with a 23-gauge needle. For every canal, one syringe was used and disposed. The experimental teeth were then irrigated with 1ml saline solution and size 35 sterile paper points were selected for sampling the bacteria from the root canals. Paper points were left in the apical portion of the canal for 1 minute and then transferred to tubes containing 5ml of BHI broth.

Tubes vertexed for 5 minutes and incubated at 37ºC for 4 days. The occurrence of broth turbidity was the indication of bacteria presence in the root canal.

For confirmation of specific presence of E.f in tubes three complementary microbiologic tests were applied as followed:

- Culturing into a blood agar plate and then gram staining.
- Catalase test
- Grow in presence of 6.5% salt and Aesculin agar hydrolysis.

Results

All samples driven from NaOCl group were negative, and all samples driven from Normal saline were positive. Because of this obvious difference, statistical analysis was not necessary.

Three complementary tests confirmed the only presence of E.f blood agar culture. Gram

staining was positive, indicating that they were cocci. Catalase test was negative, indicated that they were streptococci. Grow in presence of 6.5% salt and hydrolisis of Aescilin agar indicated that they were enterococci.

Discussion

NaOCl has been recommended as an irrigant solution in the treatment of infected root canals, because of its well-known bactericidal action.[9,10]

Even though its antibacterial effects are recognized, the exact mechanism of microbial elimination is not well elucidated.

When NaOCl is added to water, hypochlorous acid (HOCl) is formed which contain active chlorine, a strong oxidizing agent. Substantial evidence suggests that chlorine exerts its antibacterial effect by the irreversible oxidation of SH groups of essential enzymes, disrupting the metabolic functions of the bacterial cell.

Chlorine may also combine with cytoplasmic components to form N-chloro compounds, a toxic complexes which destroy the microorganism[11].

However, the first contact oxidation reactions of chlorine with bacteria may lead to the rapid killing of bacterial cells even prior to the formation of N-chloro compounds in the cytoplasm.[12]

E.f, a facultatively anaerobic gram-positive coccus, has been recovered from several oral sites.[13] This bacteria was selected for this study because of its high level of resistance to a wide range of antimicrobial agents,[14] and it is among the few facultative bacteria associated with persistent apical periodontitis.[15]

Endodontic infections with E.f usually constitute a problem with treatment because this microorganism is difficult to eliminate.[3]

Harrisan and Hand showed that diluting 5.25% NaOCl inhibits its antimicrobial properties significantly.[16]

They also reported that organic matter reduced the efficacy of 5.25% NaOCl. Nikolaus et al, showed that 50% citric acid and 5.25% NaOCl were equally effective in totally destroying obligate anaerobes that usually play an important role in the pulp and periapical pathogenesis.[17]

These two studies showed that normal saline solution has not antibacterial effectiveness.[16,17]

Sequeira et al evaluated the antibacterial effect of endodontic irrigants against eight anaerobic bacteria. They found that, these solutions from strongest to weakest are as follows:[6]

4% NaOCl, 2.5% NaOCl, 2% NaOCl, 2% chlorhexidine, EDTA and citric acid and 0.5%NaOCl.

In other study Siqueira et al found that the effectiveness of 4% NaOCl used with three irrigation methods in elimination of E.f from root canals were not different, although they were significantly more effective than the saline solution.[18]

In present study, freshly made 5.25% NaOCl was used in prepared root canals with the wide diameter in order to have enough space for acting at room temperature (21ºC).

By applying 5.25% NaOCl in prepared root canals for five minutes, there would be enough time for penetrating into all irregularities and affect on all hidden microorganisms and maximum antibacterial effect can reached.

The disinfections procedures used in this study can be repeatable in clinical conditions.

Previous studies showed that the antimicrobial effects of calcium hydroxide are best achieved if it remains in the root canal system for at least one week,[19] and one of the shortcomings of calcium hydroxide is its inability to effectively kill enterococcus species.[20]

Conclusion

By considering previous findings and the results obtained from present study it may be concluded that 5.25% NaOCl solution can be used as a “fast effect, and short term intracanal dressing”.

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