

Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal, *in vitro*

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Summary

The effectiveness of 4.0% sodium hypochlorite (NaOCl) used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal was tested *in vitro*. Root canals contaminated with *E. faecalis* were treated as follows: (i) irrigation with 2 mL of NaOCl solution and agitation with hand files; (ii) irrigation with 2 mL of NaOCl solution and ultrasonic agitation; (iii) irrigation with NaOCl alternated with hydrogen peroxide. Contaminated canals irrigated with sterile saline solution served as the control. Paper points used to sample bacteria from the root canals were transferred to tubes containing 5 mL of brain heart infusion (BHI) broth. Tubes were incubated and the appearance of broth turbidity was indicative of bacteria remaining in the root canal. There were no statistically significant differences between the experimental groups. However, NaOCl applied by the three methods tested, was significantly more effective than the saline solution (control group) in disinfecting the root canal.

Keywords: bacteria, *Enterococcus faecalis*, root canal irrigation, sodium hypochlorite.

Introduction

It has been demonstrated that bacteria and their products play an essential role in the development and perpetuation of pulpal and periradicular diseases (Kakehashi *et al.* 1965, Sundqvist 1976). Although the root canal flora is dominated by obligate anaerobic bacteria, some facultative strains, e.g. *Enterococcus fae-*

calis, have been involved in persistent infections, influencing the prognosis of the root canal treatment (Engström 1964).

Once bacteria are established in the root canal, they cannot easily be reached by the defence mechanisms of the host. 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) has been widely used as an irrigant since its introduction in endodontics by Walker in 1936. In addition to bleaching, deodorizing and tissue-dissolving properties (Gordon *et al.* 1981), NaOCl has been demonstrated to be an effective disinfectant agent (Bloomfield & Miles 1979, Block 1991).

Different irrigation regimens have been proposed to enhance the effectiveness of NaOCl in disinfecting the root canal system. Grossman (1943) suggested the alternate use of NaOCl and hydrogen peroxide (H₂O₂) for the irrigation of the root canal. This association caused effervescence, which may improve the debridement and disinfection of the root canal (Svec & Harrison 1977). Martin (1976) has proposed irrigation with NaOCl solution during ultrasonic instrumentation of the root canal system. He claimed that ultrasonic waves accelerate chemical reactions and potentiate the bactericidal efficiency of NaOCl. Studies have demonstrated that ultrasonication of NaOCl solution increases its cleaning and antibacterial effects (Cameron 1987, Sjögren & Sundqvist 1987).

Based on these premises, the purpose of this study was to compare the effectiveness of NaOCl used with three irrigation methods in eliminating *E. faecalis* from the root canal, *in vitro*.

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Materials and methods

Freshly extracted human canine teeth were selected for this study. Conventional access preparations were made and the root canals were instrumented 1 mm beyond the apical foramen with K-type files up to size 50 and then flared. Irrigation with tap water was performed during the enlarging procedures. Following root canal preparation, the enlarged 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 min at 121°C.

Pure culture of *E. faecalis* (ATCC 29212), grown in brain heart infusion broth (BHI) (Difco, Detroit, MI, USA) was used to contaminate the root canals. A suspension of *E. faecalis* cells was prepared in BHI, which had its optical density adjusted to approximately 1.5×10^8 colony-forming units mL⁻¹ by comparing its turbidity to a McFarland 0.5 BaSO₄ standard.

The plaster blocks containing the teeth were opened in a laminar air flow cabinet. Each root canal was inoculated with 10 µL of the *E. faecalis* suspension using sterile 1 mL tuberculin syringes. The blocks were then placed inside stainless steel boxes and incubated at 37°C for 24 h. After incubation, the contaminated root canals were divided into three groups according to the irrigation regimen used:

- Group 1–20 root canals were irrigated with 2 mL of a 4.0% NaOCl solution (pH = 10.0). The solution was agitated by hand with a size 30 K-type file and left in the root canal for 5 min.
- Group 2–20 root canals were irrigated with 2 mL of a 4.0% NaOCl solution. A size 15 ultrasonic file used in the Enac unit (Enac-Osada, Tokyo, Japan) was placed in the canal to its full length. The unit was activated with the file unconstrained and the solution ultrasonicated for 1 min.
- Group 3–20 root canals were irrigated using a combination of 4.0% NaOCl and 3% H₂O₂ (pH = 5.3). Canals were irrigated initially with 1 mL of NaOCl followed by 1 mL of H₂O₂. A final irrigation with 1 mL of NaOCl was performed. Twenty contaminated root canals were irrigated with 2 mL of 0.85% sterile saline solution and these served as controls. Irrigants were delivered in the canals by means of a 3-mL plastic syringe with a 23-gauge needle. The pressure applied by the operator on the syringe was sufficient to allow a flow rate of 4 mL of irrigant per minute.

The experimental teeth were then irrigated with 1 mL saline solution and size 45 sterile paper points were

selected to sample the bacteria from the root canals. Paper points were left in the canal for 1 min and then transferred to tubes containing 5 mL of BHI broth. Tubes were vortexed for 5 min and incubated at 37°C for 4 days. The occurrence of broth turbidity was indicative of bacteria remaining in the root canal.

Data obtained were analysed statistically for differences using the chi-squared test, comparing pairs of groups, with a significance level established at $P < 0.05$.

Results

Data obtained for each irrigation regimen are presented in Table 1. Eight of the previously contaminated root canals that received manual irrigation with 4% NaOCl yielded positive cultures. Bacterial growth was verified in six out of 20 root canals in which the 4% NaOCl solution was ultrasonicated. When NaOCl was used alternately with 3% hydrogen peroxide, eight of 20 cases were positive for bacterial growth. All specimens of the control group yielded positive cultures. *E. faecalis* was always recovered from all positive cultures. There were no statistically significant differences between the experimental groups ($P > 0.05$). NaOCl, used in the three irrigation methods, was significantly more effective than saline solution in disinfecting the root canal ($P < 0.05$).

Discussion

Sodium hypochlorite has been recommended as an irrigant solution in the treatment of infected root canals, because of its well-known bactericidal action (Shih *et al.* 1970, Bloomfield & Miles 1979, Foley *et al.* 1983). Even though its antibacterial effects are recognized, the exact mechanism of microbial killing is not well elucidated. When NaOCl is added to water, hypochlorous acid (HOCl) is formed, which contains 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 compo-

Table 1 Results of cultures following irrigation of the root canals contaminated with *Enterococcus faecalis*

Irrigant	Irrigating method	n	Positive cultures
NaOCl 4.0%	Manual	20	8
NaOCl 4.0%	Manual: ultrasonic agitation	20	6
NaOCl 4.0% and H ₂ O ₂ 3.0%	Manual: alternate use	20	8
Saline solution	Manual	20	20

nents to form *N*-chloro compounds, toxic complexes which destroy the microorganism (Murray *et al.* 1994). 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 (Block 1991). It appears that the disinfecting effectiveness of NaOCl increases with a decrease in pH, because the concentration of undissociated HOCl is increased. However, an alkaline pH is required for solution stability.

E. faecalis, a facultatively anaerobic Gram-positive coccus, has been recovered from several oral sites (Rams *et al.* 1992). It was selected for use in this study because it exhibits a high level of resistance to a wide range of antimicrobial agents (Heath *et al.* 1996) and it is among the few facultative bacteria associated with persistent apical periodontitis (Haapasalo *et al.* 1983). Endodontic infections with *E. faecalis* usually constitute a problem with treatment because this microorganism is difficult to eliminate (Engström 1964).

More than half of the specimens tested for each experimental regimen yielded negative cultures. Conversely, all specimens of the control group which were irrigated with sterile saline solution were positive for bacterial growth. It has not been clear whether the major effect of irrigants is to wash out bacteria through the flow and backflow of the solutions or if they have a significant antibacterial effect inside the root canal. Although the mechanical effect of irrigation has been demonstrated to reduce the number of bacteria in the root canal (Byström & Sundqvist 1983), the findings of the present study suggest that an irrigant solution which possesses antibacterial action is required to maximize the disinfection of the root canal system.

The results of the present study failed to show any significant difference between the irrigation regimens tested. Therefore, the effects observed in this study were more dependent on the NaOCl solution than on the irrigation method employed. The *E. faecalis* strain used in this study was demonstrated to be susceptible to the 4% NaOCl solution in an agar diffusion test (unpublished data), therefore it is possible that in cases which yielded positive cultures, bacterial cells located in irregularities of the root canal were protected from the lethal effects of the solution (Siqueira *et al.* 1996). A longer period may be required for the solution to reach these areas and thereby be effective. Bacteria located in these niches could also be unaffected by ultrasonication, escaping the effects of cavitation, which occurs only on bacteria in contact with the imploding bubbles (Ahmad *et al.* 1990).

This *in vitro* model has been used by other researchers

(Foley *et al.* 1983, Fegan & Steiman 1995) and allows a comparative evaluation between different irrigation regimens and/or irrigant solutions because the bacterial inoculum and the bacterial strain tested are standardized. In addition, this model enables easy handling and a more effective control of environmental contamination. However, one must bear in mind that the antibacterial effectiveness of irrigants in root canal therapy may be quite different compared to mixed cultures present in a dynamic biological system, as usually occurs *in vivo*. Thus, direct extrapolations to clinical conditions must be exercised with caution because of the obvious limitations of *in vitro* studies.

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