Chemomechanical Reduction of the Bacterial Population in the Root Canal after Instrumentation and Irrigation with 1%, 2.5%, and 5.25% Sodium Hypochlorite

José F. Siqueira, Jr., DDS, MSc, PhD, Isabela N. Rôças, DDS, Amauri Favieri, DDS, MSc, and Kenio C. Lima, DDS, MSc

Given the importance of bacteria in the development of periradicular lesions, the eradication of the root canal infection is paramount in endodontic treatment. This study evaluated the in vitro intracanal bacterial reduction produced by instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite (NaOCl) or saline solution. Root canals inoculated with *Enterococcus faecalis* were instrumented and irrigated with the solutions tested. Canals were sampled before and after preparation. After serial dilution, samples were plated onto *Mitis salivarius* agar, and the colony-forming units grown were counted. Inhibitory effects of the three NaOCl solutions on *E. faecalis* were also evaluated by means of the agar diffusion test. All test solutions significantly reduced the number of bacterial cells in the root canal ($p < 0.05$). There was no significant difference between the three NaOCl solutions tested ($p > 0.05$). Nonetheless, all NaOCl solutions were significantly more effective than saline solution in reducing the number of bacterial cells within the root canal ($p < 0.05$). The three NaOCl concentrations showed large zones of inhibition against *E. faecalis*. The results of this study suggest that regular exchange and the use of large amounts of irrigant should maintain the antibacterial effectiveness of the NaOCl solution, compensating for the effects of concentration.

It is well established that bacteria and their products play a crucial role in the development of periradicular diseases. Kakehashi et al. (1) exposed the dental pulps of conventional and germ-free rats to their own oral cavity and observed that pulp necrosis and periradicular lesions develop only in conventional rats with an oral

microbiota. In a study of monkey teeth, Möller et al. (2) demonstrated that only devitalized pulps that were infected induced periradicular lesions whereas devitalized and uninfected pulps showed an absence of any pathological changes in the periradicular tissues. Sundqvist (3) confirmed the important role of bacteria in periradicular lesions in a study using human teeth, in which bacteria were only found in root canals of pulpless teeth with periradicular bone destruction.

During endodontic treatment of pulpless teeth with periradicular lesions, the main goals of chemomechanical preparation are cleaning and disinfecting the entire root canal system and eliminating bacteria and any sources of nutrient supply such as the tissue remnants. Inadequate accomplishment of these objectives risks the short-term as well as the long-term outcome of endodontic therapy. Bacteria persisting within the root canal system are the major cause of endodontic treatment failures (4, 5).

Irrigants are used during endodontic treatment to flush out loose debris, to lubricate the dentinal walls, to dissolve organic matter in the canal, and to be antimicrobial. Cleaning and disinfecting procedures are highly dependent on the mechanical and chemical effects of the irrigants. Mechanical effects during irrigation are generated by the flow and backflow of irrigant solution in the root canal. Regardless of the type of irrigant used, the bacterial population inside the root canal is significantly reduced by the mechanical effects of irrigation (6). Nevertheless, studies have revealed that chemical compounds that possess antibacterial effects show clearly superior effectiveness in bacterial elimination when compared with saline solution (7, 8).

Several studies have generally concurred that sodium hypochlorite (NaOCl) has a broad-spectrum antimicrobial activity (7–11). It can rapidly kill vegetative bacteria, spore-forming bacteria, fungi, protozoa, and viruses (including HIV, rotavirus, HSV-1 and -2, and hepatitis A and B viruses) (11). High concentrations may be required to kill acid-fast bacilli and bacterial spores. Although NaOCl has been widely used in endodontics as an irrigant, there is no consensus regarding the ideal concentration to be used. The risk-benefit ratio should be considered during the choice of irrigant solutions.

Irrigants should ideally destroy microorganisms and neutralize their products without damaging host tissues. Therefore the desir-

able concentration should be one that possesses low toxicity and adequate antibacterial effects. Spangberg et al. (12) showed that 5.25% NaOCl was considerably stronger than necessary to eliminate bacterial strains commonly found in infected root canals. At this concentration, NaOCl was considered too cytotoxic to be routinely used in endodontic therapy. On the other hand, Thé et al. (13) evaluated the tissue response to several NaOCl concentrations in guinea pigs and observed that there was no significant difference in toxicity between NaOCl concentrations ranging from 0.9 to 8%. After injection of different solutions in the subcutaneous tissue of guinea pigs, Yesilsoy et al. (14) reported that 5.25% NaOCl was no more toxic than less concentrated NaOCl solutions and chlorhexidine gluconate.

Data concerning the antimicrobial effectiveness of different NaOCl concentrations have also revealed conflicting results. Some clinical studies (15, 16) have not found any significant difference in antibacterial effect between 0.5% and 5% NaOCl. In contrast, other studies have reported that the antibacterial effectiveness of NaOCl is significantly reduced after dilution (10, 11, 14).

The purpose of this study was to compare the in vitro intracanal bacterial reduction produced by instrumentation and irrigation with 1%, 2.5%, and 5.25% NaOCl solutions.

MATERIALS AND METHODS

Forty extracted human lower bicuspid with a single root canal, checked by radiographs, 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 20, under irrigation with tap water. Working length was established at the apical foramen. After root canal preparation, the enlarged apical foramen was sealed by epoxy resin to prevent bacterial leakage. To make both handling and identification easier, the teeth were then mounted vertically in plaster blocks and sterilized overnight by ethylene oxide gas.

Sterilized plaster blocks containing the teeth were opened in a laminar air flow cabinet. A suspension was prepared by adding 1 ml of a pure culture of *Enterococcus faecalis* (ATCC 29212), grown in Brain Heart Infusion broth (Difco, Detroit, MI) for 24 hr, to fresh Brain Heart Infusion broth. Each root canal was completely filled with the *E. faecalis* suspension using sterile 1-ml tuberculin syringes. Sterile K-type #15 files were used to carry the bacterial suspension to the entire root canal length. The blocks were then placed inside sterile plastic bags and incubated at 37°C for 24 hr.

The root canals were divided into three experimental groups accordingly to the NaOCl solution used in irrigation. In group 1, 10 root canals were irrigated with 1% NaOCl during instrumentation. In group 2, 10 other canals were irrigated with 2.5% NaOCl. In group 3, 10 root canals were irrigated with 5.25% NaOCl. All NaOCl solutions were prepared from Clorox (Clorox Corp., Oakland, CA). The control group consisted of 10 root canals irrigated with 0.85% sterile saline solution and was the same group as was used in another study (6). All root canals were prepared using Nitiflex files (Maillefer, Ballaigues, Switzerland) in an alternated rotary motions technique as described by Siqueira (17). A #25 Nitiflex file was inserted into the root canal to a point where it bound slightly and then turned clockwise with no more than one quarter rotation. It was then turned counterclockwise with light apical pressure. Counterclockwise rotation was also no more than

one quarter turn. These motions were repeated continuously until the file reached the working length. Alternated rotary motions were maintained in this position for few seconds. The file was withdrawn 1 to 2 mm, still oscillating, then replaced to the working length. This continuous oscillation associated with the up and down motions was repeated until the file was able to slide easily to the working length. Each sequentially larger file was worked in a similar fashion. Apical preparation was completed by enlargement through a #40 Nitiflex file.

The root canals were sampled before and after instrumentation. Canals were filled with sterile 0.85% saline solution, and each sample was taken by using three paper points. After initial sampling, the root canals were irrigated with 1 ml of the tested solutions. Canals were also irrigated with 1.5 ml of the tested solutions after each file size. A total volume of 7 ml of the irrigants tested was used for each tooth. Residual NaOCl was inactivated by rinsing the canals with 2 ml of a 5% sodium thiosulfate solution. A final irrigation was performed in groups 1, 2, and 3 using 1 ml of 0.85% sterile saline solution. Canals were then sampled using three paper points. Irrigant was delivered in the canals by means of a 3 ml plastic syringe with a 23-gauge needle. Each set of instruments was used to prepare no more than three root canals.

After each sampling, paper points were transferred to tubes containing 1 ml of 0.85% saline solution and vortexed for 1 min. After 10-fold serial dilutions in saline, aliquots of 0.1 ml were plated onto *Mitis salivarius* agar plates and incubated at 37°C for 48 hr. The colony-forming units grown were counted and a log transformation was performed.

Data obtained from samples taken before and after instrumentation were analyzed statistically for differences within groups using the paired *t* test and between groups by means of the ANOVA and the Tukey's test. The significance level was established at 5% ($p < 0.05$).

Inhibitory effects of the three NaOCl solutions on *E. faecalis* were also evaluated by means of the agar diffusion test. The same bacterial suspension used to contaminate the root canals was used to seed the surface of *Mitis salivarius* agar plates. Paper disks 6 mm in diameter soaked with the test solutions were placed onto the agar surface. Four agar plates were used in this experiment.

Plates were incubated aerobically at 37°C for 2 days, and the diameters of the zones of bacterial inhibition were measured and recorded for each solution tested.

RESULTS

Data obtained from the comparison between the samples taken before and after root canal preparation showed that all test solutions significantly reduced the number of bacterial cells in the root canal ($p < 0.05$).

The mean number of bacterial cells in the initial samples from the root canals irrigated with 1% NaOCl was 7.11×10^6 (range: 1.4×10^4 to 2.9×10^7). After preparation, the mean value of the number of bacterial cells decreased to 9.28×10^2 (range: 1×10^1 to 4×10^3). The mean reduction of bacteria in both absolute and log numbers were of 97.1% and 56.8%, respectively.

Instrumentation and irrigation with 2.5% NaOCl provided a decrease of 99.9% (in absolute numbers) and 59.5% (in log numbers) in the number of viable bacteria in the root canal. For this group, the means of the bacterial cell numbers at the initial and the final samples were 2.54×10^7 (range: 6.5×10^5 to 9.4×10^7) and 2.45×10^3 (range: 3×10^1 to 1×10^4), respectively.

TABLE 1. Mean values of the quantity of bacterial cells (in both absolute and log numbers) within the root canal before and after instrumentation and irrigation using the different irrigants

Solution	Initial (Mean of Absolute/Log Nos.)	Final (Mean of Absolute/Log Nos.)	Reduction (%) (Mean of Absolute Nos.)	Reduction (%) (Mean of Log Nos.)
1% NaOCl	7.11×10^6 (6.22)	9.28×10^2 (2.51)	97.1	56.8
2.5% NaOCl	2.54×10^7 (7.02)	2.45×10^3 (2.86)	99.9	59.5
5.25% NaOCl	3.56×10^6 (6.14)	2.58×10^2 (2.02)	99.8	65.9
Saline	2.7×10^6 (5.86)	1.1×10^4 (3.6)	99.6	38.3

TABLE 2. Means of the diameters of the zones of inhibition produced by the irrigants against *E. faecalis* (in mm)

Solution	Diameter (mm)
Saline	0
1% NaOCl	18.5
2.5% NaOCl	21.5
5.25% NaOCl	31

Zone size is a function of the solubility of NaOCl in agar and of the concentration gradients of the NaOCl within the agar.

Initial samples from the root canals irrigated with 5.25% NaOCl contained a mean of 3.56×10^6 (range: 4×10^4 to 1.3×10^7) bacterial cells, whereas the mean number of bacteria in the final samples was 2.58×10^2 (range: 1×10^1 to 1.1×10^3). The mean reduction for this group was 99.8% (in absolute numbers) and 65.9% (in log numbers).

Instrumentation and irrigation using saline showed a mean of bacterial reduction of 99.6% in absolute numbers and 38.3% in log numbers. The mean number of bacterial cells in the canals before and after preparations were 2.7×10^6 (range: 4×10^4 to 1.6×10^7) and 1.1×10^4 (range: 3.2×10^2 to 3.5×10^4), respectively.

Comparisons between groups using the ANOVA and the Tukey's test failed to find a significant difference between the three NaOCl solutions tested ($p > 0.05$). However, all NaOCl solutions were significantly more effective than saline solution in reducing the number of bacterial cells inside the root canal ($p < 0.05$).

Data concerning the means of bacterial cell numbers and percentages of reduction in both absolute and log values are summarized in Table 1.

The three NaOCl concentrations showed large zones of inhibition against *E. faecalis* in the agar diffusion test. Saline showed no antibacterial activity. Inhibitory effects were dependent on concentrations and could be ranked from the strongest to the weakest solutions as follows: 5.25% NaOCl, 2.5% NaOCl, and 1% NaOCl. The means of the diameters of the zones of inhibition are shown in Table 2.

DISCUSSION

E. faecalis has been commonly recovered from root canals where the endodontic treatment has failed. Molander et al. (18) examined the microbiological status of root-filled teeth with periradicular lesions and found enterococci in 32% of the investigated teeth. Under similar conditions, Möller (19) reported 29%, and Sundqvist et al. (5) 38% occurrence. Enterococci usually have the ability to survive even under unusual environmental stresses and may be extremely resistant to medications used during the endodontic therapy (17).

On the whole instrumentation with NaOCl reduced the number of bacteria in contaminated root canals by 1,000- to 100,000-fold.

However, a great variability was observed when evaluating some specimens. For example, final samples taken from one specimen from group 1 (1% NaOCl) contained 3.5-fold fewer bacterial cells than the initial sample. Another specimen from the saline group showed a bacterial reduction of 17.4-fold. This high variability caused by a few specimens might yield an erroneous comparison between groups with regard to the percentage of bacterial reduction. To confer homoscedasticity to the populations, data were then transformed in log numbers. Transformation allowed obtaining a more reliable assessment of the ability of each solution tested in reducing the number of bacterial cells within the root canals.

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. The group where 0.85% saline solution was used was the same as was used in a previous study (6). The results clearly demonstrated that the mechanical action of both instruments and irrigation could remove a significant number of bacterial cells from the main root canal. However, bacterial reduction was significantly superior when NaOCl solutions were used as irrigants. In addition to the mechanical effects, NaOCl possesses chemical effects that help in the elimination of bacteria from the root canals. One should bear in mind that in the present study the saline group received a lesser volume of irrigant than the NaOCl groups, which received additional irrigation with 2 ml of sodium thiosulfate and 1 ml of saline. Despite this difference in volume of irrigants, our findings are in agreement with other studies and support the use of antibacterial substances during the irrigation of infected root canals (7, 8).

Even though the antibacterial effects of NaOCl are recognized, the exact mechanism of microbial killing is not well elucidated. When NaOCl is added to water, hypochlorous acid is formed, which contains active chlorine, a strong oxidizing agent. Available evidence suggests that chlorine exerts its antibacterial effect by the irreversible oxidation of sulfhydryl groups of essential enzymes, disrupting the metabolic functions of the bacterial cell.

It is well known that an increase in NaOCl concentration in a solution brings a corresponding increase in the antibacterial activity as long as others factors—such as pH, temperature, and organic content—are held constant. This was confirmed in the present study when using the agar diffusion test. All test NaOCl solutions showed large zones of inhibition against *E. faecalis*. It was observed that the higher the NaOCl concentration tested, the larger the zones of inhibition produced.

Because chemomechanical preparation is a short-time procedure, it would appear that the antibacterial effectiveness of the irrigant inside the root canal might be highly dependent on its concentration. Organic material in contact with NaOCl solutions consumes available chlorine and reduces its antibacterial activity. This is evident especially in solutions with low NaOCl concentrations. Higher levels of NaOCl would tend to produce a safety

reserve for performing the desired antibacterial activity within the root canal system. Interestingly, no difference in antibacterial effects was observed between the three NaOCl concentrations used in the contaminated root canals. The results of this study suggest that frequent and copious irrigation with a weaker NaOCl solution as used herein may maintain a chlorine reserve sufficient to eliminate a significant number of bacterial cells. Frequent replenishing of the irrigants is required when one is irrigating root canals with weaker NaOCl solutions. Based on these findings, the need to use strong NaOCl solutions becomes questionable.

A considerable bacterial reduction was achieved by using the different NaOCl concentrations. Nevertheless, bacteria were never thoroughly eliminated from the root canals. The antibacterial effectiveness of the irrigant does not depend only on the cytotoxicity of the substance. To eliminate bacterial cells within the root canal system, the irrigant must reach them. Many areas of the root canal system have the potential to harbor microorganisms, thus making them inaccessible to the effects of chemomechanical preparation. Such regions include dentinal tubules, fins, and ramifications. Although NaOCl is highly effective in destroying bacteria, it does not penetrate well into these confined areas of the root canal system. In addition, it remains in the canal for only a short period of time which may limit its effectiveness to bacteria located in and nearby the main root canal. Because the method used in this study just permits evaluation of the bacteriological conditions of the main root canal, it is possible to assume that the use of a larger volume of irrigant could have increased the elimination of *E. faecalis* cells. Further studies may clarify what the ideal volume of irrigant to be used during the endodontic treatment is.

Using NaOCl in low concentrations may significantly reduce the endodontic infection, but might not consistently dissolve all pulpal remnants in a reasonable time. Nonetheless, Baumgartner and Cuenin (20) showed that NaOCl in concentrations of 1%, 2.5%, and 5.25% completely removed pulpal remnants and predentin from uninstrumented dentinal walls. Thus, it appears that low concentrations of NaOCl (e.g. 1%) retain adequate tissue-dissolving and antibacterial properties.

Although the concentration of NaOCl solution has little apparent antibacterial effects in the root canals, the efficacy of weak solutions may decrease rapidly. Hence, the antibacterial effectiveness of low concentrations of NaOCl may be improved by using larger volumes of irrigant and by frequent exchange of irrigant. Regular exchange and the use of large amounts of irrigant should maintain the antibacterial effectiveness of the NaOCl solution, compensating for the effects of concentration.

Dr. Siqueira is chairman and professor, and Drs. Rôças and Favieri are professors of Endodontics, Department of Dentistry, Estácio de Sá University,

Rio de Janeiro, Brazil. Dr. Lima is professor of Preventive Dentistry, Federal University of Rio Grande do Norte, Natal, Brazil. Address requests for reprints to Dr. José F. Siqueira, Jr., R. Heróides de Oliveira 61/601, Icaraí, Niterói, RJ, Brazil 24230-230.

References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surg* 1965;20:340-9.
2. Möller AJR, Fabricius L, Dahlén G, Öhman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. *Scand J Dent Res* 1981;89:475-84.
3. Sundqvist G. Bacteriological studies of necrotic dental pulps [Dissertation]. Umea, Sweden: University of Umea, 1976.
4. Sundqvist G, Figdor D. Endodontic treatment of apical periodontitis. In: Orstavik D, Pitt Ford T, eds. *Essential endodontology*. Oxford: Blackwell Science Ltd., 1998:242-77.
5. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg* 1998;85:86-93.
6. Siqueira JF Jr, Lima KC, Magalhães FAC, Lopes HP, Uzeda M. Mechanical reduction of the bacterial cell number inside the root canal by three instrumentation techniques. *J Endodon* 1999;25:332-5.
7. Siqueira JF Jr, Machado AG, Silveira RM, Lopes HP, Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal. *Int Endod J* 1997;30:279-82.
8. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. *Oral Surg* 1983;55:307-12.
9. Bloomfield SF, Miles GA. The antibacterial properties of sodium dichloroisocyanurate and sodium hypochlorite formulations. *J Appl Bacteriol* 1979;46:65-73.
10. Siqueira JF Jr, Batista MMD, Fraga RC, Uzeda M. Antibacterial effects of endodontic irrigants on black-pigmented gram-negative anaerobes and facultative bacteria. *J Endodon* 1998;24:414-6.
11. Rutala WA, Weber DJ. Uses of inorganic hypochlorite (bleach) in health-care facilities. *Clin Microbiol Rev* 1997;10:597-610.
12. Spangberg L, Engström B, Langeland K. Biologic effects of dental materials. III. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. *Oral Surg* 1973;36:856-71.
13. Thé SD, Maltha JC, Plasschaert AJM. Reactions of guinea pig subcutaneous connective tissue following exposure to sodium hypochlorite. *Oral Surg* 1980;49:460-6.
14. Yesilsoy C, Whitaker E, Cleveland D, Phillips E, Trope M. Antimicrobial and toxic effects of established and potential root canal irrigants. *J Endodon* 1995;21:513-5.
15. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J* 1985;18:35-40.
16. Cvek M, Nord C-E, Hollender L. Antimicrobial effect of root canal débridement in teeth with immature root. A clinical and microbiologic study. *Odontol Revy* 1976;27:1-10.
17. Siqueira JF Jr. *Tratamento das infecções endodônticas*. Rio de Janeiro: MEDSI, 1997.
18. Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. *Int Endod J* 1998;31:1-7.
19. Möller AJR. Microbial examination of root canals and periapical tissues of human teeth. *Odontol Tidskrift* 1966;74(suppl):1-380.
20. Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. *J Endodon* 1992;18:605-12.