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Antimicrobial efficacy of 4.2% sodium hypochlorite adjusted to pH 12, 7.5, and 6.5 in infected human root canals

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Objective. The purpose of this study was to determine the antimicrobial efficacy of sodium hypochlorite adjusted to pH 12, 7.5, and 6.5 in human root canals infected by *Enterococcus faecalis*.
Study design. One hundred sixty-five human single-rooted teeth were prepared and inoculated with *E. faecalis* for 48 h. Teeth were divided into 3 experimental groups according to the irrigation pattern used: group 1, 4.2% NaOCl pH 12; group 2, 4.2% NaOCl pH 7.5; and group 3, 4.2% NaOCl pH 6.5. Samples from the root canals were collected, and bacterial growth was analyzed by turbidity of the culture medium.
Results. None of the irrigating solutions used in this study demonstrated 100% effectiveness against *E. faecalis*. The antibacterial effectiveness of 4.2% NaOCl at pH 6.5 was significantly increased ($P = .03$) compared with 4.2% NaOCl at pH 12 (chi-squared test: $P < .05$).
Conclusion. Bactericidal activity of NaOCl solution is enhanced by weak acidification of 4.2% NaOCl solution at pH 6.5. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:295-298)**

Bacteria and their products are the major etiologic factors in the initiation, propagation, and persistence of periradicular periodontitis.[1,2] The outcome of root canal treatment is dependent on mechanical preparation, irrigation, microbial control, and complete obturation of the root canal system. Follow-up studies examining the success of endodontic therapy have revealed lower success rates when bacterial culture was present before root canal filling.[3] In consequence, methods used to disinfect the root canal should be capable of accessing and totally eliminating the bacteria present in all parts of the system. Sodium hypochlorite (NaOCl) is the irrigating solution most widely used. The antibacterial effectiveness of NaOCl within the root canal depends on, among others, irrigant concentration, contact time, pH, and hypochlorous acid (HClO) concentration.[4] The most important factor affecting HClO content in the NaOCl solution is pH, given that a decrease in pH increases the concentration of dissociated HClO and thus its antimicrobial effectiveness.[5] However, there is some concern regarding the chemical stability of the NaOCl solution at low pH.[6] The purpose of the present study is to compare in vitro the antimicrobial efficacy of 4.2% NaOCl adjusted to pH 12, 7.5, and 6.5 in human root canals infected by *Enterococcus faecalis*.

MATERIAL AND METHODS

One hundred sixty-five extracted permanent human single-rooted teeth were obtained and stored in saline solution. Access preparations were prepared and the patency of each canal was established by inserting an ISO size #10 K-file (Dentsply Maillefer, Baillaigues, Switzerland) until the tip emerged from the apical foramen. This length was noted and the working length (WL) of each specimen was calculated by subtracting 1 mm. The root canals were negotiated and instrumented

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to a size #20 K-type file (Dentsply Maillefer) without irrigation. The canals were then enlarged to an F3 ProTaper rotary file (Dentsply Maillefer) using the sequence recommended by the manufacturer and with the aid of an electric motor (Tecnika Vision; Dentsply Maillefer). Preparation was then completed by using a 40/04 Profile at the working length. Irrigation was performed between each rotary instrument using 1 mL 4.2% NaOCl (commercial stock solution; Henckel Ibérica, Barcelona, Spain) delivered in a 10-mL syringe. The smear layer was removed using 20% citric acid for 1 min and subsequently 4.2% NaOCl for 5 min. The apical 3 mm of each specimen was covered with Tetric Ceram (Ivoclar Vivadent, Schaan, Liechtenstein) to prevent any extrusion or leakage of material during root canal preparation and sample collection. Teeth were mounted vertically in plaster blocks, introduced in plastic boxes, and sterilized in an autoclave at 121°C for 20 min.

The bacteria *Enterococcus faecalis* (ATCC 29212) was inoculated in a Brain Heart Infusion (BHI)–agar plate (Difco, Detroit, MI). The plate was incubated aerobically at 37°C during 48 h to allow bacterial growth. One colony of *E. faecalis* was introduced in a sterile assay tube containing 5 mL BHI and was incubated for 48 h at 37°C in a shaker. Tooth specimens were inoculated with a 10-μL bacterial suspension, previously adjusted to an optical density of 0.5 at 590 nm, using sterile 1 mL tuberculin syringes and incubated for 48 h. In a previous study we had determined that a bacterial suspension of 0.5 optical density contained approximately 2×10^9 colony-forming units (CFU)/mL.

After 48 h of bacterial infection, the roots were randomly assigned to 3 experimental groups and 2 control groups, according to the irrigants to be tested. All solutions were freshly prepared, and pH was determined by using a digital pH meter (pHmeter basic 20; Crison, Barcelona, Spain). Irrigant was delivered in the canals by means of a 10-mL plastic syringe with a 23-gauge needle.

Group 1: 4.2% NaOCl solution (pH 12)

Forty-three root canals inoculated with *E. faecalis* were irrigated with 0.2 mL 4.2% NaOCl (pH 12). The solution was kept in the canal for 5 min before being rinsed out with 1 mL 5% thiosulfate ($Na_2S_2O_3$), which was then allowed to remain in the canal for 1 min, thereby inactivating the remnants of the NaOCl.

Group 2: 4.2% NaOCl solution (pH 7.5)

Forty-two root canals inoculated with *E. faecalis* were irrigated with 0.2 mL 4.2% NaOCl (pH 7.5). To maintain NaOCl at a pH of 7.5, the solution was prepared mixing 30 mL 4.2% NaOCl and 0.5 mL 99.5% acetic acid and allowed to settle for 5 min before being introduced into the canals. The solution was kept in the canal for 5 min before being rinsed out with 1 mL 5% $Na_2S_2O_3$, which was then allowed to remain in the canal for 1 min, thereby inactivating the remnants of the NaOCl.

Group 3: 4.2% NaOCl solution (pH 6.5)

Forty-three root canals inoculated with *E. faecalis* were irrigated with 0.2 mL 4.2% NaOCl (pH 6.5). To maintain NaOCl at a pH of 6.5, the solution was prepared mixing 30 mL 4.2% NaOCl and 0.9 mL 99.5% acetic acid and allowed to settle for 5 min before being introduced into the canals. The solution was kept in the canal for 5 min before being rinsed out with 1 mL 5% $Na_2S_2O_3$, which then was allowed to remain in the canal for 1 min, thereby inactivating the remnants of the NaOCl.

Positive control group

Eighteen root canals inoculated with *E. faecalis* were irrigated with 5% $Na_2S_2O_3$ for 5 min.

Negative control group

Eighteen root canals, not inoculated with *E. faecalis* but with sterile BHI, were irrigated with 5% $Na_2S_2O_3$ for 5 min.

Subsequent to $Na_2S_2O_3$ irrigation, #40 sterile paper points (Dentsply Maillefer) were introduced into the canals and maintained for 1 min for sample collection. In each specimen three paper points samples were collected. The points were individually transfered and immersed in 5 mL BHI medium and aseptically cultured in a shaker at 37°C for 72 h. After 72-h incubation, 2 observers examined the tubes in a blinded manner for the presence of turbidity. The number of positive and negative samples after treatment was recorded.

Statistical analysis

The results were analyzed statistically by the chi-squared test, and the level of significance was set at $P < .05$.

RESULTS

All positive control samples presented turbidity, revealing that $Na_2S_2O_3$ was not toxic to bacteria at the concentration used; and none of the negative controls showed turbidity, which confirmed the use of an aseptic technique throughout the experiment. Table I shows the antimicrobial efficacy of the different solutions used. The group of teeth irrigated with 4.2% NaOCl at pH 12 presented 60.5% disinfection, whereas the group

Table I. Antimicrobial activity of irrigants in human root canals infected by *Enterococcus faecalis*

Group	Samples, n	Bacterial growth, n (%)	No bacterial growth, n (%)
4.2% NaOCl, pH 12	43	17 (39.5%)	26 (60.5%)
4.2% NaOCl, pH 7.5	42	12 (27.9%)	30 (72.1%)
4.2% NaOCl, pH 6.5	43	7 (16.2%)	36 (83.8%)
Positive control	18	18 (100%)	0
Negative control	18	0	18 (100%)

Enterococcus faecalis–infected root canals were irrigated with 4.2% NaOCl at pH 12, 6.5, and 7.5 for 5 min, and the disinfection capacity was determined after 72 h of incubation at 37°C by recording bacteria growth observed as turbidity.

treated with 4.2% NaOCl at pH 7.5 presented 72.1% disinfection, and the group treated with 4.2% NaOCl at pH 6.5 presented 83.8% disinfection. There was a significant increase in the disinfecting capacity of the group at pH 6.5 with respect to the group at pH 12 ($P = .03$), however, the difference was not significant between the groups at pH 12 and at pH 7.5 ($P = .4$) or between the groups at pH 7.5 and at pH 6.5 ($P = .27$).

DISCUSSION

The antimicrobial efficacy of intracanal medicaments has been long investigated using different experimental models. Various authors have used in vivo models,[7-10] and others have used in vitro models, such as infected human teeth[11,12] or infected bovine teeth.[13-15] Similar studies have been carried out in test tubes[16-19] or agar plates,[20-23] but they do not accurately replicate the conditions in human teeth, because anatomic hindrances in teeth do not permit direct contact between the antimicrobial substances and the microorganisms.[19,23] Disagreement among authors may be due to differences in experimental procedures, the bacterial strains tested, incubation time, and conditions or growth media used.

Enterococcus faecalis was chosen for several reasons: It occurs primarily in retreatment scenarios; it has been extensively used in experimental work in endodontics, including antiseptic susceptibility[24]; it survives in root canals as a single organism without the support of other bacteria[8]; and it is an easy-to-grow bacteria in the laboratory.[14] Bacteria were allowed to grow in the canals for 48 h at 37°C, because the physiological state of bacteria influences the outcome of the antimicrobial treatment. It has been shown that starved bacteria (48 h growth) are more resistant to different types of stress compared with growing cells.[25]

Bacteriologic sampling is another important step that varies among the different methodologies.[26] In the present study, bacterial samples were taken with sterile paper points. The use of paper point technique has the advantage that it can be performed in vitro and in vivo.[11] On the other hand, bacteriologic sampling with paper points is limited, because only the microorganisms that are present in the main root canal can be sampled, and the ones that are located inside the dentin tubules are inaccessible. The method of obtaining dentinal samples using burs of different diameters can evaluate the presence of bacterial cells inside the dentinal tubules; however, it was not included in this study.

Sodium hypochlorite is still the best-known irrigant, owing to its solvent action on pulp tissues, its marked bactericidal action, and its action as a lubricant. However, many studies have shown that, despite correct instrumentation and correct use of the irrigant, it is almost impossible to obtain a canal system free from bacteria and pulp residues.[7] Gomes et al.[23] reported that 5 min of 4% NaOCl irrigation was enough to eliminate *E. faecalis*. In the present study, 4.2% NaOCl at pH 12 showed a 60.5% disinfection against *E. faecalis*. This result agrees with others, who obtained 60% and 65% disinfection.[26,27]

Acidic NaOCl solution was prepared by adding acetic acid. Kuroiwa et al.[5] reported that acetic acid is the most desirable acid for the preparation of acidic NaOCl solution because of its bactericidal activity and safety. Mixtures with other acids (i.e., lactic acid, citric acid) consume available chlorine and reduce capacity for bactericidal activity of the disinfectant,[5,28] although acetic acid had no effect on available chlorine.

Results confirm that a pH reduction of the NaOCl solution improves its antimicrobial activity, showing significant differences at pH 6.5. Likewise, Bremer et al.[29] reported an increased effectiveness of the pH-adjusted solutions (pH 6.5) against *Listeria monocytogenes*. The antimicrobial effectiveness of NaOCl depends on the pH and the hypochlorous acid concentration.[4,30] Fukuzaki[30] reported an increase of hypochlorous acid concentration at pH 6, with 95% of hypochlorous acid at pH 6.5 and 45% at pH 7.5. At pH 7.5, there is an insufficient increase of hypochlorous acid to compensate for a drop in pH, and that may be the reason there is no significant difference between groups 1 (pH 12) and 2 (pH 7.5) as reported in the present study. The main problem of decreasing the pH of NaOCl is the instability of the resulting solution. Presumably, buffer solutions could be added to keep the pH level constant. Tissue-dissolving capacity and cytotoxicity tests of pH-adjusted NaOCl solutions remain to be shown in further studies.

In conclusion, bactericidal activity of 4.2% NaOCl solution is enhanced by weak acidification of NaOCl solution to 6.5 pH.

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