Brazilian Dental Journal (2014) 25(5): 416-419
http://dx.doi.org/10.1590/0103-6440201300049
ISSN 0103-6440

Effect of a Surfactant on the Antimicrobial Activity of Sodium Hypochlorite Solutions

Marcos Rodolfo Bolfoni[1], Marcelo dos Santos Ferla[1], Otávio da Silva Sposito[1], Luciano Giardino[2], Rogério de Castilho Jacinto[1], Fernanda Geraldes Pappen[1]

[1]Department of Semiology and Clinics, Dental School, UFPel - Federal University of Pelotas, Pelotas, RS, Brazil
[2]Department of Endodontics, Dental School, University of Torino, Italy

Correspondence: Profa. Dra. Fernanda Geraldes Pappen, Rua Gonçalves Chaves 457, 507, 96015-560 Pelotas, RS, Brasil. Tel: +55-53-3335-6741, +55-53-8403-3599. e-mail: ferpappen@yahoo.com.br

The objective of the present study was to evaluate the antimicrobial activity of sodium hypochlorite (NaOCl) associated with a surfactant. Seventy single-rooted extracted human teeth were inoculated with *Enterococcus faecalis,* and incubated for 21 days (37 °C). The groups were distributed according to the irrigation solution used during root canal preparation: 5%, 2.5% and 1% NaOCl; 5%, 2.5% and 1% Hypoclean®, a solution containing a surfactant (cetrimide) associated with NaOCl. Three microbiological samples were collected from each tooth: S1 - before instrumentation; S2 - immediately after instrumentation; and S3 - after a seven-day period. Data were submitted to ANOVA and Tukey test with 5% significance level. The results showed that immediately after root canal preparation (S2), *E. faecalis* was eliminated in all the experimental groups. However, after 7 days (S3), only the groups in which Hypoclean was used, remained contamination-free, including Hypoclean associated with 1% NaOCl, while the root canals irrigated with 1% NaOCl only, presented the highest percentage of bacterial growth. In conclusion, the addition of surfactant increased the antimicrobial activity of 1% NaOCl to levels similar to 5% NaOCl.

Key Words: antimicrobial effect, cetrimide, sodium hypochlorite, surfactant.

Introduction

A range of instrumentation techniques and irrigation regimens is available to accomplish a successful endodontic treatment. The use of chemical solutions during root canal preparation is an important tool in the decontamination of the root canal system. Endodontic irrigants are infiltrated into the canal system to flush out loose debris, kill microbes, remove microbial byproducts, dissolve organic tissues, and remove the smear layer (1).

Sodium hypochlorite (NaOCl) in concentrations ranging from 0.5% to 5.25%, is the most widely used solution in the root canal treatment, as it appears to satisfy most of the requirements for an ideal root canal irrigating solution (2,3), e.g., it presents high organic tissue-dissolving ability (2), and an antimicrobial wide-spectrum activity (3), especially in concentrations higher than 2.5%. However, it has been shown that 2.5% and 5.25% NaOCl are more toxic to the periapical tissues than 1% NaOCl (4).

Despite its high pulp-dissolving capacity and its antimicrobial activity, NaOCl has a high surface tension (48.90 mJ/m^2), which may limit its penetration into the irregularities of the root canal and the depth of dentinal tubules (5). Surface tension may be reduced by using heat or adding chemicals known as surfactants (6). Therefore, Hypoclean® (Ogna Laboratori Farmaceutici, Milan, Italy) - a solution of 5% NaOCl with added surfactants (cetrimide and polypropylen glycol), with low surface tension (29.13 MJ/m^2) – has been evaluated as an endodontic irrigating solution (7). Hypoclean showed an increased capacity to kill bacteria compared with the 5.25% NaOCl solution (5), and an increased pulp-dissolving capacity (8).

The fact that Hypoclean has 5.25% NaOCl in its formulation may be of concern. Some authors advocate that 0.5% and 1% NaOCl solutions are safer than NaOCl at higher concentrations, as 1% NaOCl has been shown to produce cytotoxic effects and inflammatory reactions when reaching the periapical tissue (4,9). On the other hand, NaOCl at low concentrations presents a low capacity to disinfect (10) and to remove debris from the root canal system (11). Nevertheless, the addition of detergents like cetrimide and polypropylene glycol may increase its antimicrobial capacity in root canal irrigation, while maintaining the low cytotoxicity of the solution. The aim of this study was to evaluate the effect of a surfactant on the antimicrobial activity of different concentrations of NaOCl solutions, in order to determine whether the addition of surfactants to NaOCl is able to optimize the disinfection of the root canal system.

Material and Methods

Specimens Standardization

Seventy sound extracted permanent human anterior

teeth with single straight canals were used in this study. The study was independently reviewed and approved by the Federal University of Pelotas (UFPel) Ethics Committee (document no.182/2010).

After immersion in 1% NaOCl the teeth were cleaned and stored in distilled water under refrigeration. The number of specimens per group was calculated using SigmaStat software, version 3.5 for Windows. Dental crowns of all specimens were cut at the level of the cementoenamel junction with a diamond disk.

To obtain standardization of the specimens, the root canals were instrumented in all their extent until an apical diameter corresponding to a #40 K-file. The canals were irrigated with 1 mL of 5% NaOCl between each instrument. After instrumentation, the root canals were irrigated with 5% NaOCl for 5 min, and then with 17% EDTA for 5 min, to obtain open dentinal tubules free of smear layer, so that the bacterial infection had access to the dentinal tubules. Finally, the teeth were irrigated with 5 mL distilled water.

Subsequently, the apical area was sealed with light cured composite resin Opallis (FGM Dental Products Ltd., Joinville, SC, Brazil), and root surfaces were externally sealed with two coats of cyanoacrylate, except in the cervical region.

The specimens were randomly assigned to separate Eppendorf tubes. Each tooth was fixed into this tube using chemically activated acrylic resin. The set containing the tooth included in acrylic resin was autoclaved prior to the experiments.

Contamination with Enterococcus faecalis

The microbiological procedures were performed in aseptic environment in a laminar flow chamber (VecoFlow Ltda, Campinas, SP, Brazil). Cultures of pure *Enterococcus faecalis* (ATCC 29212) were reactivated in Tryptic Soy Broth - TSB (Difco, Detroit, MI, USA) for 48 h. The bacteria were inoculated into Tryptic Soy Agar plates - TSA (Difco, Detroit, MI, USA) and incubated at 37 °C for 24 h. A bacterial suspension was prepared in sterile water, equivalent to 3 X 108 colony forming units (CFU)/mL. The adjustment of the optical density of the suspension was made in a spectrophotometer (SP22 - 325 to 1,000 nm Bioespectro, PR, Brazil) at a 405 nm wavelength .

The culture medium (TSB) was mixed with the bacterial suspension at a ratio of 1:1 and the root canals were infected with 20 µL of this suspension. A sterile cotton swab was dipped in TSB and placed at the entrance of the root canals. The specimens were maintained at 37 °C and relative humidity for 21 days, with the TSB being renewed every three days.

After 21 days, all root canals were collected to confirm the contamination (S1). Three sterilized absorbent paper points #35 (Tanari Industrial Ltda., Manacapuru, AM, Brazil) were used per tooth. Each paper point was kept in the root canal for 1 min and transferred to Eppendorf tubes containing 1 mL sterile distilled water. Each tube was agitated in vortex for 30 s (56 AP; Phoenix, Araraquara, SP, Brazil). Aliquots of 20 µL of ten-fold dilutions (up to 10-4) were seeded in triplicate into TSA medium. The plates were incubated at 37 °C for 48 h. Bacterial growth was detected by counting the number of CFU/mL of *E. faecalis*.

Solutions

Pure NaOCl and NaOCl associated to cetrimide and polypropylene glycol (Hypoclean) were obtained as 5.25% stock solutions (Ogna Laboratori Farmaceutici, Milan, Italy). The solutions were kept at 4 °C following the manufacturer's recommendations and brought to room temperature before use. The 5.25% solutions were diluted in distilled water immediately before use in order to obtain 5%, 2.5%, and 1% solutions. Distilled water was used as control.

Experimental Groups

The roots were fixed in Eppendorf tubes and randomly divided into seven groups (n=10) according to the used irrigating solution: groups irrigated with NaOCl in different concentrations: 5%, 2.5% and 1%, groups irrigated with NaOCl associated with cetrimide and polypropylene in the same concentrations: 5%, 2.5% and 1%, and the control group containing distilled water.

All root canals were instrumented to a #55 K-file, and 2 mL of respective irrigating solution was used between each instrument. The planned gradual decline was performed up to a #70 K-file. The total time for instrumentation and maintenance of irrigating solution was standardized at 10 min per specimen. At the end, the root canals were irrigated with 2 mL sterile distilled water.

Sampling and Microbiological Analysis

Immediately after instrumentation, a second microbiological collection was performed (S2). For this purpose, sterile #55 absorbent paper cones were used, following the same procedures described for the initial collection. Next, the root canals were filled with TSB medium and a sterile cotton swab culture was placed at the entrance of the root canals. Microplates containing the specimens were closed again and incubated at 37 °C. After 7 days, a third microbiological sampling was conducted (S3) following the same procedures of the first two collections.

Statistical Analysis

The results were analyzed using SPSS for Windows version 17.0. After checking the normal distribution of the results, comparison between the experimental groups was performed by applying ANOVA and Tukey's multiple

comparison test. The level of significance was set at 5%.

Results

The results showed that after instrumentation (S2), the tested irrigating solutions, except for the distilled water used in the control group, were able to totally eliminate the infection from the root canals.

After 7 days, when a new collection was performed (S3), only in the groups in which NaOCl was associated with surfactants (Hypoclean®) the root canals remained free from contamination. The lowest bacterial reduction occurred in the control group, followed by 1% NaOCl Group (Table 1), while 1% NaOCl + cetrimide showed 100% bacterial reduction after 7 days.

Discussion

Considering that both manual and mechanized instrumentation techniques leave many areas of the root canals untouched by the instruments, it is paramount to use irrigation solutions that present satisfactory antimicrobial activity during the whole biomechanical preparation (1). This study confirmed the antimicrobial activity of NaOCl, and demonstrated that the addition of surfactants optimizes the ability of lower concentrations of NaOCl in disinfecting the root canals.

E. faecalis is a facultative anaerobic microorganism, easily grown, with a high clinical relevance and ability to establish and survive in the absence of other bacteria, and is commonly isolated in resistant endodontic infections (12). This microorganism also presents greater resistance to antibacterial agents when compared to other species (13), justifying its use in the present study. The complexity of complete bacterial eradication of the root canal and its influence on the failure of endodontic treatment system is widely discussed in the literature (1,14,15). One factor that contributes to this difficulty is the inhibition of the antibacterial activity of the disinfectants by the chemical environment of the root canal (16,17). The validity of direct contact tests and agar diffusion tests has been questioned, since these methods do not allow interactions between components of dentin tissue, bacteria and disinfectants (18). Thus, the evaluation of the antimicrobial capacity of irrigating solutions and medicaments used during root canal treatment should preferably be performed according to methodologies that reproduce the clinical conditions found in infected root canals.

Table 1. Mean values (S.D.) of bacterial reduction of root canals evaluated among the different experimental groups

Irrigating solution	Bacterial reduction after preparation (%)	Bacterial reduction after 7 days (%)
5% NaOCl	100[a]	99.65 (0.32)a
2.5% NaOCl	100[a]	96.42 (1.82)[a]
1% NaOCl	100[a]	53.02 (22.52)[b]
5% Hypoclean®	100[a]	100[a]
2.5% Hypoclean®	100[a]	100[a]
1% Hypoclean®	100[a]	100[a]
Control group	99.22 (0.33)[b]	4.18 (27.58)[c]

Immediately after instrumentation, all substances presented 100% of bacterial reduction, which may be related to the mechanical effect of instrumentation, as even the control group, despite the statistical difference, showed a high percentage of bacterial reduction (99.22%). The true effect of the different solutions was observed in the third collection (S3). After 7 days, the bacterial reduction in the control group was 4.18%, meaning that bacteria viable in dentinal tubules recolonized the root canal. Also, after 7 days, bacteria could be found in all NaOCl groups, with a statistically lower bacterial reduction to 1% NaOCl, which may be explained by the lack of NaOCl residual antimicrobial activity (19,20).

Lower concentrations of NaOCl have lower antimicrobial activity, and consequently lower capacity for disinfecting root canals (20). This fact was confirmed in this study, where 7 days after biomechanical preparation, the bacterial reduction in root canals irrigated with 1% NaOCl was about 53%, while 2.5% and 5% NaOCl solutions removed more than 95% of initial infection of root canals. Despite the lower antimicrobial activity of 0.5% and 1% NaOCl, these solutions have been indicated because of their recognized low cytotoxicity, safer to periapical tissues (21,22).

After 7 days, however, only the groups irrigated with Hypoclean remained free of contamination. Similar antimicrobial activity was observed in the groups in which 5% and 2.5% NaOCl solutions were used, while these groups were statistically superior to 1% NaOCl. In both evaluation periods, the control group had the lowest percentage of bacterial reduction. These results may reflect the superior diffusion capacity of NaOCl solution when associated with surfactants, because of the surface tension reduction (23). Therefore, the penetration of these irrigating solutions in the main canals, side canals, and within dentinal tubules may be optimized during mechanical preparation (23). Furthermore, the absence of bacterial contamination even 7 days after the mechanical preparation may also be the result of a possible residual effect of cetrimide. This possible residual effect is expected, since cetrimide is a quaternary ammonium, as chlorhexidine, with similar chemical behavior (24).

Cetrimide, even at concentrations between 0.01% and 0.04%, can increase the antibacterial effect of some antibiotics (24). Recent studies have demonstrated that this agent increases the antimicrobial activity of irrigating

solutions like chlorhexidine and NaOCl (7,25). The addition of detergents such as polypropylene and cetrimide used in Hypoclean® may be an alternative for irrigation of root canals, because the surfactants provide lower surface tension to the solutions, therefore optimizing penetration of the irrigating solution in dentinal tubules, with consequent increase in their disinfecting power.

In conclusion, after 7 days of mechanical preparation, the combination of NaOCl solution with cetrimide and polypropylene increases the antimicrobial activity of 1% NaOCl to values similar to 5% NaOCl and a more biocompatible concentration of the solution.

Resumo

O objetivo da presente pesquisa foi avaliar a atividade antimicrobiana de hipoclorito de sódio (NaOCl), associado a um tensoativo. Setenta dentes humanos monorradiculares extraídos foram inoculados com *Enterococcus faecalis* e incubados durante 21 dias (37 °C). Os grupos foram distribuídos de acordo com a solução irrigadora utilizada no preparo do canal: hipoclorito de sódio a 5%, 2,5% e 1%; Hypoclean® a 5%, 2,5% e 1% - uma solução contendo um surfactante (cetrimida) associado com NaOCl. Três amostras microbiológicas foram coletadas de cada dente: S1 - antes de instrumentação; S2 - imediatamente após a instrumentação; e S3 - após um período de sete dias. Os dados foram submetidos à análise de variância e teste de Tukey com 5% de nível de significância. Os resultados mostraram que imediatamente após o preparo do canal radicular (S2), o *E. faecalis* foi eliminado em todos os grupos experimentais. No entanto, após 7 dias (S3), apenas os grupos em que se utilizou Hypoclean permaneceram livres de contaminação, incluindo Hypoclean 1%, enquanto que os canais radiculares irrigados apenas com hipoclorito de sódio 1% apresentaram a mais elevada percentagem de crescimento bacteriano. Em conclusão, a adição de surfactante aumentou a atividade antimicrobiana de 1% de NaOCl a níveis semelhantes aos do NaOCl 5% .

Acknowledgements

The author Marcos Rodolfo Bolfoni received scholarships during this study from Programa de Educação Tutorial – PET, Odontologia, UFPel. FAPERGS (0903932/2009) also supported this study.

References

1. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J 2014; 216:299-303.
2. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. J Endod 2004;30:785-787.
3. Virtej A, MacKenzie CR, Raab WH, Pfeiffer K, Barthel CR. Determination of the performance of various root canal disinfection methods after in situ carriage. J Endod 2007;33:926-929.
4. Pashley EL, Birdsong NL, Bowman K, Pashley DH. Cytotoxic effects of NaOCl on vital tissue. J Endod 1985;11:525-528.
5. Giardino L, Ambu E, Becce C, Rimondini L, Morra M. Surface tension comparison of four common root canal irrigants and two new irrigants containing antibiotic. J Endod 2006;32:1091-1093.
6. Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M. Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. J Endod 2010;36:1558-1562.
7. Palazzi F, Morra M, Mohammadi Z, Grandini S, Giardino L. Comparison of the surface tension of 5.25% sodium hypochlorite solution with three new sodium hypochlorite-based endodontic irrigants. Int Endod J 2012;45:129–135.
8. de Almeida LH, Leonardo NG, Gomes AP, Giardino L, Souza EM, Pappen FG. Pulp tissue dissolution capacity of sodium hypochlorite combined with cetrimide and polypropylene glycol. Braz Dent J 2013;24:477-481.
9. Navarro-Escobar E, González-Rodríguez MP, Ferrer-Luque CM. Cytotoxic effects of two acid solutions and 2.5% sodium hypochlorite used in endodontic therapy. Med Oral Patol Oral Cir Bucal 2010;15:e90-e94.
10. Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ. Comparative study of the antimicrobial efficacy of chlorhexidine gel, chlorhexidine solution and sodium hypochlorite as endodontic irrigants. Braz Dent J 2007;18:294-298.
11. Baratto-Filho F, de Carvalho JR Jr, Fariniuk LF, Sousa-Neto MD, Pécora JD, da Cruz-Filho AM. Morphometric analysis of the effectiveness of different concentrations of sodium hypochlorite associated with rotary instrumentation for root canal cleaning. Braz Dent J 2004;15:36-40.
12. Poptani B, Sharaff M, Archana G, Parekh V. Detection of *Enterococcus faecalis* and *Candida albicans* in previously root-filled teeth in a population of Gujarat with polymerase chain reaction. Contemp Clin Dent 2013;4:62-66.
13. Misuriya A, Bhardwaj A, Bhardwaj A, Aggrawal S, Kumar PP, Gajjarepu S. A comparative antimicrobial analysis of various root canal irrigating solutions on endodontic pathogens: An in vitro study. J Contemp Dent Pract 2014;15:153-160.
14. Farzaneh M, Abitbol S, Lawrence HP, Friedman S. Treatment outcome in endodontics - the Toronto Study. Phase II: initial treatment. J Endod 2004;30:302-309.
15. Marquis VL, Dao T, Farzaneh M, Abitbol S, Friedman S. Treatment outcome in endodontics: the Toronto Study. Phase III: initial treatment. J Endod 2006;32:299-306.
16. Haapasalo H, Sirén E, Waltimo T, Ørstavik D, Haapasalo M. Inactivation of local root canal medicaments by dentine: *in vitro* study. Int Endod J 2000;33:126–131.
17. Pappen FG, Qian W, Aleksejuniene J, Leonardo R de T, Leonardo MR, Haapasalo M. Inhibition of sodium hypochlorite antimicrobial activity in the presence of bovine serum albumin. J Endod 2010;36:268-271.
18. Haapasalo M, Udnaes T, Endal U. Persistent, recurrent and acquired infection of the root canal system post-treatment. Endod Topics 2003;6:29-56.
19. Mohammadi Z, Abbott PV. Antimicrobial substantivity of root canal irrigants and medicaments: a review. Aust Endod J 2009;35:131-139.
20. Virtej A, MacKenzie CR, Raab WH, Pfeiffer K, Barthel CR. Determination of the performance of various root canal disinfection methods after in situ carriage. J Endod 2007;33:926-929.
21. Simbula G, Dettori C, Camboni T, Cotti E. Comparison of tetraacetylethylendiamine + sodium perborate and sodium hypochlorite cytotoxicity on L929 fibroblasts. J Endod 2010;36:1516-1520.
22. Hidalgo E, Bartolome R, Dominguez C. Cytotoxicity mechanisms of sodium hypochlorite in cultured human dermal fibroblasts and its bactericidal effectiveness. Chem Biol Interact 2002;139:265-282.
23. Mohammadi Z, Mombeinipour A, Giardino L, Shahriari S. Residual antibacterial activity of a new modified sodium hypochlorite-based endodontic irrigation solution. Med Oral Patol Oral Cir Bucal 2011;16:588-592.
24. El-Nima. The synergism between cetrimide and antibiotics against *Pseudomonas aeruginosa*. Zentralbl Bakteriol Mikrobiol Hyg A 1984;258:120-127.
25. Portenier I, Waltimo T, Orstavik D, Haapasalo M. Killing of *Enterococcus faecalis* by MTAD and chlorhexidine digluconate with or without cetrimide in the presence or absence of dentine powder or BSA. J Endod 2006;32:138-141.

Received April 4, 2014
Accepted September 19, 2014