

Antimicrobial Activity of Different Concentrations of NaOCl and Chlorhexidine Using a Contact Test

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The purpose of this study was to analyze the *in vitro* antimicrobial activity of sodium hypochlorite (1% and 5%) and chlorhexidine (0.12%, 0.5% and 1%). Bacterial samples (ATCC) of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum* were submitted to a contact test. Solutions were evaluated at different time intervals: immediately, 5 min, 15 min, and 30 min after contact and repeated 10 times. The results of the contact test showed that 0.12% chlorhexidine did not eliminate *E. faecalis* at any time interval, while 0.5% and 1% chlorhexidine and 1% and 5% sodium hypochlorite did. These results permit us to conclude that to obtain better antimicrobial activity, chlorhexidine in a concentration greater than 0.12% should be used.

Key Words: antimicrobial activity, sodium hypochlorite, chlorhexidine, endodontics.

INTRODUCTION

The harmful effect of microorganisms present in pulp and periapical pathologies has been studied since 1894, when Miller (1) proved the presence of bacteria in the interior of root canals and observed their importance in the etiology of such alterations. This was then confirmed by posterior studies (2,3).

It is a well-known fact that only the mechanical action of instruments is not capable of promoting satisfactory cleansing due to the complexity of internal dental anatomy (apical deltas, lateral canals, accessory canals, etc.) and because there is no direct contact of instruments with all walls of the root canal system. Therefore, the physical and chemical actions of irrigating solutions become necessary.

Among these solutions, sodium hypochlorite has been used frequently. Its therapeutic importance in endodontics was initially suggested by Walker in 1936

(4) promoting a double action: the dissolution of necrotic tissues (attributed to its high pH) and its germicide property, related to the formation of hypochlorite acid by the liberation of chloride ions from its solution. The antibacterial action of hypochlorite acid occurs by the oxidation of bacterial enzymes which lead to the disorganization of their metabolism (4).

Many solutions have been tested with the intent of encountering an irrigating solution which permits the substitution of sodium hypochlorite because of its toxicity (4). Among these solutions, chlorhexidine gluconate is acknowledged as a wide spectrum antimicrobial agent. It is unique in its ability to bind to oral tissue for extended periods, from which it is released slowly (substantivity), and it is relatively nontoxic. However, it does not have the capacity to dissolve tissues (5).

The ideal irrigating solution must be an antimicrobial agent that does not cause toxic effects to periapical tissues while in contact with them. There-

fore, its toxic action must be selective (6).

Thus, the objective of this study was to evaluate *in vitro* the antimicrobial activity of different concentrations of sodium hypochlorite and chlorhexidine on strict and facultative anaerobic microorganisms at different time intervals, using the contact test.

MATERIAL AND METHODS

The following irrigating solutions were tested: 1% sodium hypochlorite, 5% sodium hypochlorite, 0.12% chlorhexidine digluconate, 0.5% chlorhexidine digluconate, 1% chlorhexidine digluconate. All solutions were produced by Bakton Microbiology Ltda. (Fundação Bio-Rio, Cidade Universitária, RJ).

All bacterial strains used in this experiment were obtained from the American Type Culture Collection® (ATCC, Rockville, MD, USA) and maintained frozen at -70°C, in 10% skim milk (Difco Laboratories, Detroit, MI, USA), containing 5% of glicerol (Merck). Three strains of facultative anaerobic microorganisms were selected: *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), and two strains of strict anaerobic microorganisms: *Porphyromonas gingivalis* (ATCC 33277) and *Fusobacterium nucleatum* (ATCC 25568). Both facultative and strict anaerobic microorganisms were cultivated and maintained in proper atmosphere and medium.

All the bacteria samples were centrifuged and washed three times for the removal of possible residues of culture media. After this step, the bacteria cells were again suspended in Hank's balanced saline solution (HBSS), containing 0.1% bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO, USA). To standardize the strict and facultative bacterial suspensions, the samples were diluted and counted to obtain a suspension of approximately 10⁹ colony forming units per millimeter of suspension (cfu/ml).

Table 1. Antimicrobial effect of chlorhexidine (CHX) and NaOCl solutions on *E. faecalis*.

Time after contact	0.12% CHX	0.5% CHX	1% CHX	1% NaOCl	5% NaOCl
Immediate	+	+	-	-	-
5 min	+	-	-	-	-
15 min	+	-	-	-	-
30 min	+	-	-	-	-

Ten experiments were carried out for each irrigating solution following the methodology recommended by Cremieux and Fleurete (7), which is a contact test to evaluate the antimicrobial activity of two disinfectants. A bacteria suspension is mixed with a disinfectant solution in a fixed volume and, after determining time of contact, the antimicrobial activity is verified through the cultivation of the resultant mixture in an adequate nutritive medium. One millimeter of bacterial suspension (10⁹ cfu/ml) was centrifuged in microtubes. The supernatant was discarded and 1 ml of each test solution was poured over the sediment (pellet). After homogenization, 50-µl aliquots of the facultative microorganism suspensions were transferred to 5 ml of tripticasein soy broth (TSB), and strict anaerobic organisms to 5 ml of thioglycolate broth (Merck). This was done at different times after contact with the test solution: immediately, 5 min, 15 min and 30 min. Subsequently, 10 µl of bacterial dilutions were uniformly applied to Müeller-Hinton agar and incubated in a bacteriological kiln for 48 h at 37°C for facultative bacteria. Ten microliters of diluted solutions containing strict anaerobic bacteria were uniformly applied on the surface of supplemented blood-agar petri dishes and were immediately incubated in jars in anaerobiosis for 7 days at 37°C. The antimicrobial activity was evaluated after this period through the verification of presence or absence of bacterial growth for each specimen, solution and time tested. Controls for analysis of bacteria viability of strict and facultative anaerobic bacteria were also included and the samples were incubated with saline solution for similar times. The tubes, from which the 50-µl aliquots of suspensions were taken, were included in the experiment as a control of bacterial growth.

RESULTS

Table 1 presents the antimicrobial activity of chlorhexidine solutions and NaOCl in relation to *E. faecalis* using the contact test. Solutions tested eliminated *S. aureus*, *F. nucleatum*, *E. coli* and *P. gingivalis* at all time periods. The 0.12% chlorhexidine solution did not eliminate *E. faecalis* at any time. The 1% chlorhexidine solution and

1% and 5% sodium hypochlorite eliminated all the microorganisms tested independent of time.

DISCUSSION

Byström and Sundqvist (8) demonstrated that the surgical-chemical preparation of root canals with the aid of a non-antiseptic irrigating solution reduced the number of bacteria by 50%. However, when 0.5% sodium hypochlorite was used 80% of bacteria was eliminated. The use of chemical substances during surgical-chemical preparation of the root canal is important for the cleansing of the root canal system (9).

In the present study, we used strains that best represented endodontic infections and that were good models to be tested for antimicrobial sensitivity. Three facultative anaerobic bacteria strains were tested (*E. faecalis*, *S. aureus* and *E. coli*) because these are present in all phases of the development of an infection in root canals (10,11). Two strict anaerobic strains were also tested (*P. gingivalis* and *F. nucleatum*) because these are most prevalent in necrotic pulp (3,12).

Although sodium hypochlorite is the most clinically used irrigating solution, there is no agreement about its optimum concentration (8). Spangberg et al. (13) tested many irrigating solutions in both *in vitro* and *in vivo* studies, and they concluded that an ideal solution is that which combines both maximum antimicrobial effect with minimum toxicity.

Chlorhexidine solution presents a wide spectrum antimicrobial action, substantivity (14) and is relatively nontoxic (15). However, it does not dissolve organic material (16).

Addition of 0.1% bovine serum albumin to Hank's balanced saline solution was performed during the preparation of bacteria suspensions, as an organic charge for the neutralization of the solutions (7). This neutralization is desirable so that the solution does not continue to react after the tested time and, thus, prevent false negative results.

In a similar study, Ohara et al. (17) evaluated the antimicrobial activity of 0.2% chlorhexidine and 5.25% sodium hypochlorite *in vitro* after 1, 15, 30 and 60 min and after 1 h of contact for many strains including *P. gingivalis* and *F. nucleatum* and reported total elimination of bacteria.

Our results for NaOCl are in agreement with those of D'Arcangelo et al. (18), who used the same

concentration of ufc/ml. Even with the lowest concentration tested (0.2%), chlorhexidine eliminated all microorganisms tested including *E. faecalis*, *E. coli*, *P. gingivalis* and *F. nucleatum* during the shortest time period of 10 min. It is not possible to declare any opposition between the results presented by these authors and the present study, considering that we tested chlorhexidine at smaller concentrations and contact times (0 and 5 min). It was these times for which the solution was ineffective. Another factor to be considered was the association of chlorhexidine to Cetrimide®, which D'Arcangelo et al. (18) consider a good tensoactive agent with consistent antimicrobial properties.

Silva et al. (19) sought to compare the antimicrobial effectiveness of different concentrations of sodium hypochlorite (1 and 2.5%) and chlorhexidine (0.12 and 2%), in a similar *in vitro* test, after 10, 20 and 30 min of contact, analyzing many strains including *S. aureus*, *E. faecalis* and *E. coli*. They also reported total elimination of these microorganisms at all times and concentrations tested.

In another study in which a similar methodology was used, Shih et al. (20) tested different dilutions of Clorox® with suspensions of *Streptococcus faecalis* and *S. aureus*. Bacteria elimination was demonstrated after 30 s of contact with non-diluted Clorox and dilutions of 1:10, 1:100 and 1:1000. In this same study, another experiment was carried out simulating a clinical procedure using extracted teeth artificially contaminated by the same microorganisms. However, negative cultures after immediate instrumentation were only obtained after the use of non-diluted Clorox. The authors explained this result by the presence of pulp tissue and by the limited contact of irrigating solutions in the interior of the root canal and, perhaps, by the fact that the neutralizer used in this contact test was not effective.

According to our results, we conclude that chlorhexidine solutions must be used in a concentration greater than 0.12% to obtain a larger antimicrobial spectrum and should be used when contact between the irrigating solution and the periapical region is inevitable such as with an open apex, perforation or paraendodontic surgery. It should also be used in cases of allergy to NaOCl and as a medicament between appointments taking advantage of its quality of substantivity. However, 1% and 5% sodium hypochlorite continue to be the first choice for irrigation.

RESUMO

O objetivo do presente trabalho foi avaliar *in vitro* a atividade antimicrobiana do hipoclorito de sódio (1% e 5%) e da clorexidina (0,12%, 0,5% e 1%). Cepas (ATCC) de *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Porphyromonas gingivalis* e *Fusobacterium nucleatum* foram submetidas ao teste por contato. As amostras foram analisadas em diferentes intervalos de tempo: imediatamente, 5 minutos, 15 minutos e 30 minutos após o contato com cada solução e repetidas 10 vezes. Os resultados demonstraram que a solução de clorexidina a 0,12% não foi capaz de eliminar as amostras de *E. faecalis* em nenhum intervalo de tempo testado enquanto que a clorexidina a 0,5% e 1% eliminaram todas as cepas testadas assim como as 2 concentrações de hipoclorito de sódio. Estes resultados nos permitem concluir que para se obter melhores respostas quanto a atividade antimicrobiana, a solução de clorexidina deve ser utilizada numa concentração superior a 0,12%.

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Accepted October 11, 2002