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Antibacterial Effectiveness of Peracetic Acid and Conventional Endodontic Irrigants

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This study evaluated the *in vitro* antibacterial activity of conventional and experimental endodontic irrigants against *Enterococcus faecalis*. The following substances were evaluated by direct contact test: 2.5% sodium hypochlorite (NaOCl); 2% chlorhexidine (CHX); 1% peracetic acid. After different contact periods (30 s, 1, 3, and 10 min), a neutralizing agent was applied. Serial 10-fold dilutions were prepared and plated onto tryptic soy agar (TSA) and the number of colony-forming units *per* milliliter (CFU/mL) was determined. Sterile saline was used as a negative control. Both 2.5% NaOCl and 2% CHX eliminated *E. faecalis* after 30 s of contact. Peracetic acid reduced the bacterial counts by 86% after 3 min and completely eliminated *E. faecalis* after 10 min. These results allow us to conclude that 1% peracetic acid is effective against *E. faecalis*, despite its slower action compared with 2.5% NaOCl and 2% CHX.

Key Words: sodium hypochlorite, chlorhexidine, peracetic acid, root canal irrigants, Endodontics.

INTRODUCTION

Pulpal and periapical diseases are inflammatory conditions of microbial etiology (1). Therefore, one of the main goals of endodontic treatment is the elimination of infection in the root canal system in order to obtain an environment conducive to healing of apical periodontitis (2). Persistence of microorganisms after treatment is the main cause of failure in endodontic therapy (3). *Enterococcus faecalis* is a Gram-positive bacterium capable of invading dentin tubules and binding to collagen. Moreover, this microorganism has shown resistance to several irrigating solutions and medications used in Endodontics (4).

Root canal preparation *per se,* comprising instrumentation and irrigation, is unable to fully eradicate endodontic infection (5) because in many cases bacteria can propagate to areas of difficult access, such as lateral canals, apical deltas and dentin tubules (6). New intracanal irrigants and medications have been evaluated in order to reach higher success rates in endodontic therapy.

Sodium hypochlorite (NaOCl) still is the most commonly used irrigant. It is recognized for its antimicrobial activity, tissue-dissolving and detergent actions, and capacity to neutralize toxic products (7). A solution of 2.5% NaOCl is generally used when treating teeth with necrotic pulp and apical periodontitis (8). NaOCl solutions at higher concentrations have a greater irritating effect on the apical and periapical tissues (9).

Chlorhexidine gluconate (CHX) is a potent antiseptic, widely used for chemical control of dental plaque. The use of CHX as an endodontic irrigant has also been indicated due to its broad spectrum of antimicrobial activity and substantivity (10). The efficacy of 2% CHX against *E. faecalis* has been demonstrated both *in vitro* and *in vivo* (11,12). However, its main disadvantage, the lack of organic matter-dissolving ability (13), may compromise its cleaning effectiveness (14).

The search for alternative irrigating solutions has focused on substances with antibacterial effect and capacity to clean dentin surfaces. Peracetic acid, used in hospitals and in the food industry, is one of these

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substances. A recent *ex vivo* study revealed its ability to remove the smear layer (15). Its excellent antimicrobial effect is not affected by the presence of organic matter (16,17). These properties justify preliminary studies on the use of peracetic acid as root canal irrigants.

A large number of substances have been tested against *E. faecalis*, with conflicting results (4,11,18). Thus, investigations leading to an irrigating agent capable of eliminating *E. faecalis* from root canals and dentin tubules are desirable. This study compared the *in vitro* antibacterial activity of peracetic acid and conventional endodontic irrigants against *E. faecalis*.

MATERIAL AND METHODS

All microbiological assays were conducted in a laminar flow chamber (VecoFlow Ltda, Campinas, SP, Brazil). The following substances were tested: 2.5% NaOCl, 2% CHX and 1% peracetic acid. Sterile saline was used as a negative control and to determine the initial counts of viable bacterial colonies.

The antibacterial effect was evaluated using a standard *E. faecalis* strain (ATCC 29212). *E. faecalis* subcultures were plated onto tryptic soy agar - TSA (Difco Laboratories Inc., Detroit, MI, USA) prior to each test. Streak plating was performed in order to obtain isolated colonies and to verify the purity of the material.

Prior to the test, a bacterial suspension was prepared in sterile saline and adjusted spectrophotometrically (Femto, São Paulo, SP, Brazil) to the density of 3×10^7 colony-forming units *per* milliliter (CFU/mL) using a 600 nm wavelength. The suspension was used within 60 min after adjusting.

Precision pipettes were used to transfer 1.45 mL of each solution into 2 mL Eppendorf test tubes. Next, a 50 µL aliquot of *E. faecalis* suspension was added to the tube and the mixture was agitated for 30 s (Vortex AP 56; Phoenix, Araraquara, SP, Brazil). Contact periods were 30 s, 1, 3, and 10 min. Then, serial 10-fold dilutions up to 10^{-5} were made, and 100 µL aliquots of the mixture were transferred to a second test tube containing 0.9 mL neutralizing agent. The neutralizers used were: for 2.5% NaOCl, 1% sodium thiosulfate; for 2% CHX, 0.5% lecithin + 1% Tween 80; and for 1% peracetic acid, 1% sodium thiosulfate + 1% Tween 80. The contents of the first dilution were homogenized and 100 µL were transferred to a third test tube, identical to the second tube, containing 0.9 mL of neutralizing solution. The fourth, fifth and sixth tubes contained 0.9 mL of sterile saline.

Finally, three 20 µL aliquots of each dilution were distributed onto TSA plates, which were then incubated at 37ºC for 48 h in aerobiosis. The readings determined the mean number of CFU in the 3 areas of bacterial growth on each plate, for the sample dilutions numbered from 5 to 50. From these results, the number of CFU/mL was calculated for each contact period between the irrigant and the bacterial suspension.

All experiments were carried out in triplicate. The values obtained were expressed as the mean percentage of colonies still viable after each experimental period.

RESULTS

Both 2.5% NaOCl and 2% CHX completely eliminated *E. faecalis* after 30 s of contact. For peracetic acid, elimination only occurred after 10 min.

Table 1 shows the percentage of viable microbial cells after exposure to the tested irrigants.

DISCUSSION

The direct contact test, despite not being able to fully reproduce the clinical conditions observed in endodontic infections, provides some insights and allows comparison between the substances, without external factors that might interfere with their antimicrobial action. Both *ex vivo* and *in vivo* tests are important, especially since it is known that components of the tooth, such as the dentin, collagen, and other proteins, can inhibit the activity of medications and disinfectants commonly used in endodontics (19).

An important aspect of the methodology we used is the previous evaluation of the carry-over effect of the irrigants used. Accordingly, the first and second tubes containing serial dilutions received a neutralizing solution to prevent transfer of irrigant residues into the

Table 1. Viable cells (%) after exposure to the irrigants.

Groups	Contact periods			
	30 s	1 min	3 min	10 min
2.5% NaOCl	0	0	0	0
2% CHX	0	0	0	0
1% peracetic acid	96.6	72	14	0
Saline (control)	100	100	100	100

culture medium, thus assuring that the irrigation solution would not interfere with bacterial growth for longer than the predetermined experimental period (20).

Previous studies have demonstrated the efficacy of 2% CHX against *E. faecalis* (11,12), but the results in the literature are conflicting, probably because of methodological differences (11,18). We observed similar antibacterial effect for 2% CHX and 2.5% NaOCl. Both were able to eliminate *E. faecalis* at the minimum experimental period of 30 s.

Several concentrations of peracetic acid - 0.2% (16), 2.25% (15), and 10% (13) - have been evaluated, and dentin erosion has been shown to occur when higher concentrations are used (15). A low concentration (1%) was used in the present study, reducing *E. faecalis* counts by 86% after 3 min of contact and eliminating this microorganism after 10 min. These results show the antimicrobial potential of peracetic acid and increase the interest in developing studies to evaluate this substance using different methodologies and concentrations.

Considering the methodology employed and the results obtained in this study, it may be concluded that 1% peracetic acid is effective against *E. faecalis*, despite its slower action compared with 2.5% NaOCl and 2% CHX, which eliminated this microorganism within only 30 s. Future studies to evaluate this substance using different methodologies and concentrations would be opportune.

RESUMO

Este estudo avaliou, *in vitro*, a atividade antibacteriana de soluções irrigadoras convencionais e experimentais sobre *Enterecoccus faecalis*. As seguintes substâncias foram avaliadas por teste de contato direto: hipoclorito de sódio (NaOCl) a 2,5%, clorexidina (CHX) a 2%, ácido peracético a 1%. Após diferentes períodos de contato (30 s, 1, 3 e 10 min), um agente neutralizante foi empregado. Diluições decimais seriadas foram realizadas e semeadas em placas de *tryptic soy agar* (TSA). O número de unidades formadoras de colônia por mililitro (UFC/mL) foi determinado. Solução salina foi utilizada como controle negativo. Ambos, NaOCl a 2,5% e CHX a 2%, eliminaram *E. faecalis* após 30 s de contato. O ácido peracético reduziu a contagem bacteriana em 86% após 3 min e eliminou completamente *E. faecalis* após 10 min. Estes resultados permitem concluir que o ácido peracético a 1% é efetivo sobre *E. faecalis*, apesar de sua ação mais lenta quando comparado ao NaOCl a 2,5% e CHX a 2%.

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