
Antimicrobial effects of various endodontic irrigants on selected microorganisms

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Abstract

Ayhan H, Sultan N, Çirak M, Ruhi MZ, Bodur H. Antimicrobial effects of various endodontic irrigants on selected microorganisms. *International Endodontic Journal*, 32, 99–102, 1999.

Aim This study was undertaken to determine the antimicrobial effect of various endodontic irrigants against six selected microorganisms

Methodology *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus salivarius*, *Str. pyogenes*, *Escherichia coli* and *Candida albicans* were included in the study. Pre-sterilized Whatman paper discs, 6 mm in diameter and soaked with the test solution, were prepared and placed onto the previously seeded agar Petri plates. Each plate was incubated aerobically. A zone of inhibition was recorded for each plate and the results were analysed statistically.

Results 5.25% NaOCl was effective against all test microorganisms with a substantial zone of inhibition. Saline was always ineffective. Decreased concentration of NaOCl significantly reduced its antimicrobial effect. Cresophene showed a significantly larger ($P < 0.05$) average zone of inhibition compared to the other experimental irrigants. Alcohol had smaller but not significantly different zones of inhibition than chlorhexidine.

Conclusions 5.25% NaOCl was superior in its antimicrobial abilities compared with other irrigants used. A reduced concentration of NaOCl (0.5%) resulted in significantly decreased antimicrobial effects. When compared with 21% alcohol, 0.5% NaOCl and 2% chlorhexidine, paramonochlorophenol (cresophene) showed a greater antimicrobial effect.

Keywords: endodontic irrigants, microorganisms, root canal irrigation

Introduction

Various factors have been reported which contribute to the failure of root canal treatment, notably the presence of persistent intraradicular infection. (Shih *et al.* 1970, O'Hara *et al.* 1993, Barnard *et al.* 1996, Barbosa *et al.* 1997, White *et al.* 1997). Microbial control within the pulpal tissue and root canal space is a prerequisite for the prevention and treatment of pulpal and periapical breakdown. Unfortunately, it is difficult to eliminate all microorganisms and organic debris from the root canal system, regardless of the

irrigant and instrumentation (Ringel *et al.* 1982, Baumgartner *et al.* 1984, Smith & Wayman 1986).

The ideal irrigant should be strongly antimicrobial, but not toxic to the periapical tissues if exuded through the apex (Smith & Wayman 1986, Yeşilsoy *et al.* 1995). Delany *et al.* (1982) compared the antimicrobial effects of irrigation on root canal flora with 0.2% chlorhexidine gluconate solution and saline solution (0.9% sodium chloride). They showed that 0.2% chlorhexidine gluconate solution has significantly greater antimicrobial effect than saline solution when used as an irrigant in the root canal treatment of teeth with necrotic pulps.

Sodium hypochlorite (NaOCl) has been widely recommended as an irrigant for the chemomechanical debridement of root canals because of its solvent activity for both necrotic and vital tissues and its

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ability to act as an antiseptic (Jeansonne & White 1994, Barnard *et al.* 1996).

Spangberg *et al.* (1973) tested various potential irrigants both *in vivo* and *in vitro*. They reported that the most desirable solution was provided with the irrigant which combined maximal antimicrobial effect with minimal toxicity. None of those tested met this requirement. They reported that 5.25% NaOCl was considerably stronger than necessary to kill the bacteria commonly present in the root canal. However, at this concentration, NaOCl was highly toxic and irritating. Furthermore, 0.5% NaOCl dissolved the necrotic tissue but had no effect on *Staphylococcus aureus*.

The purpose of this study was to test the antimicrobial effects of some various root canal irrigants used against selected microorganisms often found in infected root canals.

Materials and methods

The six root canal irrigants used in this study were:

- 1 5.25% solution of NaOCl (Clorox, Oakland, CA, USA)
- 2 0.5% solution of NaOCl (Labcco Inc., Houston, TX, USA)
- 3 2.0% solution chlorhexidine gluconate (Procter and Gamble, Cincinnati, OH, USA)
- 4 Alcohol (21%) (Laboratoire SPAD, Quetigny, France)
- 5 Cresophene (paramonochlorophenol, 30%; thymol, 5%; dexamethasone, 0.1%) (Septodont, Saint-Maur, France)
- 6 Sterile physiological saline solution (Baxter, Deerfield, IL, USA).

Physiological saline solution was used as the negative control and 5.25% NaOCl was used as the positive control.

The microorganisms tested in this study were *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus salivarius*, *Streptococcus pyogenes*, *Escheria coli* and *Candida albicans*. Microorganisms were subcultured on both brain heart infusion agar (BHI) (Becton Dickinson, Cockeysville, USA) in 5% sheep blood (for *Strep. salivarius* and *Strep. pyogenes*) and BHI agar (for the others). These were stored for 24 h at 37°C. Several colonies from the plates were taken to BHI broth (Difco, Detroit, MI, USA) which was incubated for 35 h at 37°C. Their density was adjusted to McFarland 0.5 by adding 0.85% NaCl. BHI agar or BHI agar with 5% sheep blood plate was swabbed with the bacteria suspension. Paper discs (6 mm diameter) were soaked with 15 µL of the chemotherapeutic test solutions and placed on the plates, which were incubated at 37°C for 24 h. Zones of inhibition were measured across the diameter with a transparent ruler and recorded. The tests were repeated five times for all strains. Statistical analysis was carried out using an analysis of variance, with Duncan's test of multiple comparisons. Significance was determined at $P < 0.05$.

Results

Table 1 shows the antimicrobial activity of tested materials.

The results of the positive control (5.25% NaOCl) showed that it was effective against all test microorganisms with a substantial zone of inhibition. In contrast, the negative control (saline) was always ineffective. Decreased concentrations of NaOCl (0.5%) resulted in significantly decreased antimicrobial effects ($P < 0.05$) compared with 5.25% NaOCl.

In the present study, the group using alcohol showed smaller zones of inhibition than chlorhexidine, but the differences were not statistically significant ($P > 0.05$).

Table 1 Antimicrobial activity of the tested materials (mean zone of inhibition in mm)

	<i>n</i>	5.25% NaOCl	0.5% NaOCl	Chlorhexidine gluconate	Alcohol	Cresophene	Saline
<i>Strep. pyogenes</i>	5	45	2.5	20	20	38	0
<i>C. albicans</i>	5	61	7	24	18	44	0
<i>Strep. salivarius</i>	5	40	5.6	19	17	39	0
<i>Staph. aureus</i>	5	55	4.5	22	18	28	0
<i>E. coli</i>	5	47	5.3	26	19	30	0
<i>E. faecalis</i>	5	52	4.7	22	17	26	0
Average		50	4.93	22.16	18.1	34.16	0

Cresophene showed a significantly larger average zone of inhibition as compared with the other experimental irrigants ($P < 0.05$).

Discussion

Antimicrobial activity of an *in vitro* environment depends upon the pH of the substrate in plates, sensitivity of the drug, the amount of the bacteria inoculated, incubation time, and the metabolic activity of the microorganisms. Furthermore, the antimicrobial properties of an agent are directly related to its ability to diffuse in agar. (Updengraff & Chaug 1977). On the other hand, the duration of effectiveness of the drug, temperature, contamination and possible leakage of the agent into the mouth must be considered whilst working *in vivo* (Demirtola & Alaçam 1977, Updengraff & Chaug 1977). The microorganisms used in this study were relevant because they are part of the endodontic microbiological flora (Sundqvist 1992, Siqueira *et al.* 1997).

The use of the best possible irrigant during chemo-mechanical preparation is of great importance. The ideal irrigant should combine antimicrobial action and a capacity to dissolve organic and inorganic remnants (Georgopoulou *et al.* 1994).

Sodium hypochloride has been recommended as an irrigant solution in the treatment of infected root canals, because of its well-known bactericidal action (Nikolaus *et al.* 1988, Siqueira *et al.* 1997, Türkün & Cengiz 1997). Even though its antibacterial effects are recognized, the exact mechanism of microbial killing is not well elucidated. When NaOCl is added to water, hypochlorous acid (HOCl), which contains active chlorine, a strong oxidizing agent, is formed. Substantial evidence suggests that chlorine exerts its antibacterial effect by the irreversible oxidation of -SH groups of essential enzymes, disrupting the metabolic functions of the bacterial cell. Chlorine may also combine with cytoplasmic components to form *N*-chloro compounds, toxic complexes which destroy the microorganism. However, the first contact oxidation reactions of chlorine with bacteria may lead to the rapid killing of bacterial cells even prior to the formation of *N*-chloro compounds in the cytoplasm (Siqueira *et al.* 1997).

Byström *et al.* (1983) compared the antimicrobial effects of irrigation on canal flora with 0.5% NaOCl and saline solution (0.9% sodium chlorite). They found that 0.5% NaOCl solution showed a greater antimicrobial effect than saline solution when used

as a root canal irrigant. The cytotoxicity of NaOCl is reduced at lower concentrations, but dilution impairs its tissue dissolution, canal debridement and antimicrobial properties (McComb & Smith 1975, Harrison & Hand 1981). In the present study, the microbiological test results showed that 5.25% NaOCl was an effective agent against all the microorganisms tested. However, when the NaOCl was diluted to a clinically relevant level (0.5%), it was much less effective.

From the results of previous investigations, it seemed reasonable to assume that chlorhexidine gluconate solution might be an effective endodontic irrigant. It was postulated that the chlorhexidine gluconate solution, because of its ability to be adsorbed and released by dental tissues, would disinfect the tissues and then, by sustained release of chlorhexidine gluconate into the root canal, maintain a root canal devoid of microorganisms. This greater reduction in microorganisms would therefore result in a higher success rate for root canal therapy (Parsons 1980, Ringel *et al.* 1982).

Delany *et al.* (1982) tested chlorhexidine gluconate (2.0%) in an *in vitro* study using extracted teeth. They reported that it can be an effective antibacterial agent when used as an endodontic irrigant. They also recommended chlorhexidine as an intracanal inter-appointment dressing. The results of the present study also indicated that 2.0% chlorhexidine is an effective irrigant for endodontic use.

Although Yeşilsoy *et al.* (1995) found that 11.6% alcohol was ineffective against most microorganisms when used as an irrigant in root canals, the present study showed that higher concentrations of alcohol (21%) proved to be an active antimicrobial agent with the test microorganisms used.

Cresophene, which consists of powerful bactericides combined with a corticosteroid, has a number of properties which are particularly effective in the disinfecting of root canals. The percentage of paramonochlorophenol in cresophene is 30–45%. *In vitro* and *in vivo* studies showed that it is effective against many microorganisms found in infected root canals (Harrison & Madonia 1971, Taylor *et al.* 1976). Another agent, timol, found in cresophene is an antimicrobial and antifungal agent. It has been used in endodontic treatments combined with other antiseptics (Demirtola 1977).

Although cresophene was found to be an alternative irrigant, one still has to be cautious because of the cytotoxic, and possible carcinogenic, mutagenic and teratogenic properties of this material.

Conclusion

NaOCl (5.25%) was superior in its antimicrobial abilities when compared with other irrigants used. A decreased concentration of NaOCl (0.5%) resulted in significantly decreased antimicrobial effects. When compared with 21% alcohol, 0.5% NaOCl and 2% chlorhexidine, cresophene was found to have greater antimicrobial effects. Chlorhexidine (2%) and alcohol (21%) were effective irrigants for endodontic use.

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