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In vitro antibacterial activity of different endodontic irrigants

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Abstract – *Aim*: The objective of this study was to compare *in vitro* the antibacterial activity of Tetraclean (mixture of doxycycline, citric acid and polypropylene glycol), Niclor 5 (5.25% sodium hypochlorite solution), Clorexidin (0.2% chlorhexidine and 0.2% cetrimide solution) and hydrogen peroxide 12 volumes on three endodontic pathogens associated with primary endodontic infections. *Enterococcus faecalis*, *Streptococcus mutans and Staphylococcus aureus* strains were selected to evaluate the antibacterial activity of endodontic irrigants by the agar disc-diffusion test. *Material and methods*: Paper discs were saturated with each one of the test solutions (at room temperature and preheating at 50°C) and placed onto culture agar-plates preadsorbed with bacterial cells and further incubated for 24 h at 37°C. The growth inhibition zones around each irrigant were recorded and compared for each bacterial strain. Kruskal–Wallis and Mann–Whitney tests were applied to compare the various groups. *Results*: At room temperature, Tetraclean showed significantly higher inhibition of bacteria growth than all other irrigants tested. Preheating at 50°C significantly increased growth inhibition for all the groups tested. At 50°C, hydrogen peroxide 12 volumes and Tetraclean showed significantly higher efficacy than all other irrigants tested. *Conclusions*: 50°C-preheated hydrogen peroxide 12 volumes and Tetraclean showed highest inhibition of the bacterial growth.

Successful root canal treatment is based on cleaning, shaping and sealing the root canal system (1); the main objective of root canal therapy is the elimination of microorganisms from the root canal system and the prevention of recontamination after treatment (2–5). The complex anatomy of the root canal system limits the mechanical action of endodontic instruments, and the use of chemical solution with antibacterial activity is recommended: endodontic irrigant solutions are used to facilitate the debridement and disinfection of the root canal space and are considered to be essential for successful endodontic treatment (6–10). Mechanical preparation cannot effectively eliminate bacteria from the root canal system (11); thus, canal irrigants are needed to aid in the debridement of the canals (11, 12). The ideal properties of an endodontic irrigant are organic and inorganic tissue solvent, antimicrobial action, non-toxic, low surface tension and lubrification (1). Currently, no solution meets all those requirements. Hydrogen peroxide is an irrigation solution, which is an active agent that affects a wide range of organisms such as bacteria, yeasts, fungi, viruses and spores. The antibacterial effect of hydrogen peroxide involves hydroxyl radicals that are a potent oxidant. They can react with macromolecules, such as membrane lipids and DNA, thus resulting in bacterial death (13). Chlorhexidine gluconate has a substantial antimicrobial action (7, 14–16) and has relatively low toxic effects, but it does not dissolve organic tissue; it acts by adsorbing onto the cell wall of the microorganism and causing leakage of the intracellular components (17). EDTA is considered a moderate antibacterial agent, and it is appreciated for its ability to chelate hard tissue as decalcifying agent (18, 19). Sodium hypochlorite is the most commonly used endodontic irrigant; advantages to NaOCl include the mechanical flushing of debris from the canal, the ability of the solution to dissolve vital and necrotic tissue, the antimicrobial action and the lubricating action; in addition, it is inexpensive and readily available (1). But, even if it is a highly effective antimicrobial agent, it does not remove the smear layer from the dentin walls (20–26). Free chlorine in NaOCl dissolves necrotic tissue by breaking down proteins into amino acids; to obtain this effect, concentrations ranging from 0.5% to 5.25% have been recommended (27). Increasing the temperature of hypochlorite irrigant (from 22 to 50°C), significantly increases its tissue-dissolving ability and its bactericidal action (28). Recently, a new irrigating solution has been developed: Tetraclean (Ogna Laboratori Farmaceutici, Muggiò, Italy) is a mixture of doxycycline (50 mg per 5 ml), citric acid and polypropylene glycol (29). Tetraclean, is used as a final rinse during the root canal preparation; it is able to eliminate microorganisms and smear layer

in dentinal tubules of infected root canals with a final 4-min rinse (29, 30).

In literature, there are no studies that compared antibacterial activity, with and without preheating, of Tetraclean, Niclor 5, Cloreximid and hydrogen peroxide 12 volumes against different microorganisms associated with primary endodontic infections.

Therefore, the objective of this study was to compare *in vitro*, by the agar disc-diffusion test, the antibacterial activity of Tetraclean (mixture of doxycycline, citric acid and polypropylene glycol), Niclor 5 (5.25% sodium hypochlorite solution), Cloreximid (0.2% chlorhexidine and 0.2% cetrimide solution) and hydrogen peroxide 12 volumes against three different microorganisms. The null hypothesis of the study was that there is no significant difference in antibacterial activity among the various irrigants.

Material and methods

The microbial species used in this study were as follows: *Enterococcus faecalis* (ATCC 19433), *Streptococcus mutans* (CCUG 35176) and *Staphylococcus aureus* (Cowan 1 ATCC 13301).

All strains were cultured in Brain Heart Infusion (BHI; Difco, San Jose, CA, USA) supplemented with 10% (v/v) horse serum (Oxoid, Garbagnate Milanese, Italy). These cultures, used as source for the experiments, were statically incubated at 37°C under aerobic conditions and reduced at a final density of 1×10^{10} cells per ml as determined by comparing the OD_{600} of the sample with a standard curve relating OD_{600} to cell number. The agar disc-diffusion test of the endodontic irrigants was studied on BHI agar (1.8% w/v) plate containing 10% horse serum. Each microbial strain was evaluated against the following irrigants: Tetraclean (mixture of doxycycline, citric acid and polypropylene glycol), Niclor 5 (5.25% sodium hypochlorite solution), Cloreximid (0.2% chlorhexidine digluconate and 0.2% cetrimide solution), hydrogen peroxide 12 volumes (0.3% hydrogen peroxide) and NaCl saline solution (control). The same manufacturer (Ogna Laboratori Farmaceutici) prepared all the endodontic irrigants. The microbial strains were evaluated against the same four groups of irrigants, after 50°C preheating in a syringe warming device (Keydent, Vaterstatten, Germany). A calibrated electronic thermometer with micro-chip (Testo AG, Lenzkirk, Germany) was used to verify irrigants temperature (28).

Agar disc-diffusion test

In the agar plate-diffusion test, plates of agarized Mueller–Hinton medium (Oxoid, Cambridge, UK) were incubated for 20 min at 37°C with 3 ml of an overnight suspension of each bacterial strain (1×10^8 colony forming unit per ml, CFU ml^{-1}) grown in BHI with 10% horse serum. Sterile paper discs (diameter of 6-mm) (Oxoid, Cambridge, UK) were then saturated with 40 μl of each irrigant and then aseptically transferred to the agar plate previously incubated with bacteria. The plates were incubated at 37°C and examined after 24 h. The size of the resulting zones of inhibition was measured (mm) by an independent observer with sliding callipers and calculated as follows: size of growth inhibition zone = (diameter halo−diameter specimen)/2. The results were recorded in terms of the average diameter of growth inhibition zone. Fifteen parallel samples per each irrigant were tested.

Statistical analysis

Growth inhibition zones were calculated. Kruskal–Wallis test was applied to determine significant differences among the various groups. Mann–Whitney test was applied as *post hoc*. Significance was predetermined at $P < 0.05$. Statistical analysis was performed with STATA 7.0 Software (Stata Corp., Station College, TX, USA).

Results

Kruskal–Wallis test reported significant differences among agar diffusion tests of the various irrigants ($P < 0.001$). Mann–Whitney test showed differences among various irrigants as reported in Tables 1, 2 and 3. Tables 1, 2 and 3 show mean zones of microbial growth inhibition of irrigants after 24 h (at room temperature and preheating at 50°C) against *E. faecalis*, *S. mutans and S. aureus*, respectively. Figures 1, 2 and 3 show mean zones of microbial growth inhibition created by the tested irrigants for each bacterial strain. At room temperature, Tetraclean showed significantly higher inhibition of bacterial growth than all other irrigants tested ($P < 0.05$). Preheating at 50°C significantly increased growth inhibition for all the groups tested ($P < 0.001$). At 50°C, hydrogen peroxide 12 volumes and Tetraclean showed significantly higher efficacy than all other irrigants tested ($P < 0.01$). When compared

Table 1. Growth inhibition diameters (mm) of endodontic irrigants against *Enterococcus Faecalis* (the results were recorded in terms of the average diameter of inhibition zone); SD between parentheses

Irrigants	37°C	50°C
Tetraclean	0.9 (0.12)	1.37 (0.15)
Niclor 5	0.6 (0.17)	0.95 (0.21)
Cloreximid	0.2 (0.07)	0.55 (0.12)
Hydrogen peroxide 12 volumes	0.2 (0.1)	1.75 (0.19)
NaCl saline solution	0 (0)	0 (0)

Table 2. Growth inhibition diameters (mm) of endodontic irrigants against *Streptococcus mutans* (the results were recorded in terms of the average diameter of inhibition zone); SD between parentheses

Irrigants	37°C	50°C
Tetraclean	1.75 (0.11)	1.9 (0.09)
Niclor 5	0.45 (0.05)	0.75 (0.18)
Cloreximid	0.35 (0.08)	0.83 (0.21)
Hydrogen peroxide 12 volumes	0.25 (0.1)	2.15 (0.22)
NaCl saline solution	0 (0)	0 (0)

Table 3. Growth inhibition diameters (mm) of endodontic irrigants against *Staphylococcus aureus* (the results were recorded in terms of the average diameter of inhibition zone); SD between parentheses

Irrigants	37°C	50°C
Tetraclean	1.4 (0.1)	1.65 (0.09)
Niclor 5	0.4 (0.11)	0.59 (0.11)
Cloreximid	0.25 (0.09)	0.58 (0.15)
Hydrogen peroxide 12 volumes	0.15 (0.04)	1.5 (0.08)
NaCl saline solution	0 (0)	0 (0)

with other irrigants, the negative control (0.9% NaCl saline solution) was ineffective with all the bacterial strains both at room temperature and after preheating at 50°C.

Discussion

The null hypothesis of the present study has been rejected. Significant differences were found among the various endodontic irrigants. The result of this study indicated that different root canal irrigants showed varying level of effectiveness in the growth inhibition of the bacterial strains tested.

The antibacterial study on agar disc-diffusion test is a well-established technique (31, 32). However, the antibacterial property of an endodontic irrigant is directly related to its ability to diffuse in agar plate. The bacterial strains chosen for this study were relevant, because *E. faecalis* and *S. mutans* are part of the endodontic microbiological flora, whereas *S. aureus* is considered to be a contaminant and was tested as a reference (10, 24, 25). Brown & Doran (6) showed that hydrogen peroxide was able to dislodge necrotic tissue and dentin debris

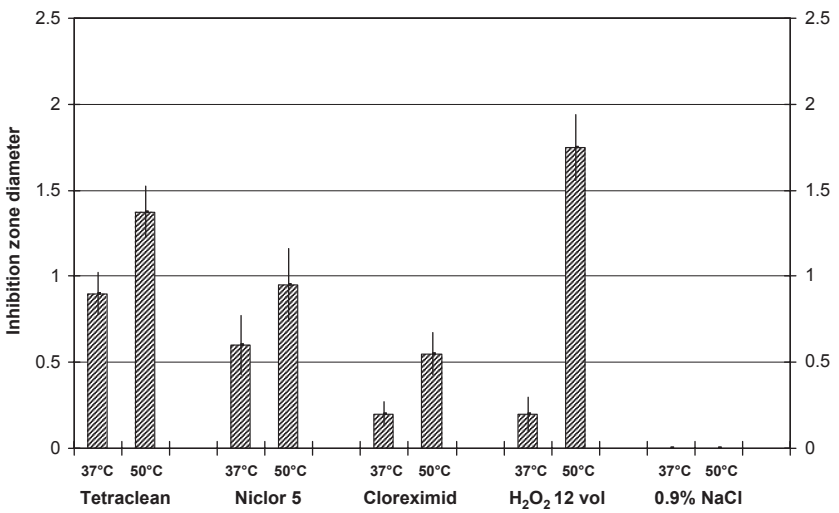

Fig. 1. Enterococcus faecalis – inhibition zone diameter (mm) of different groups.

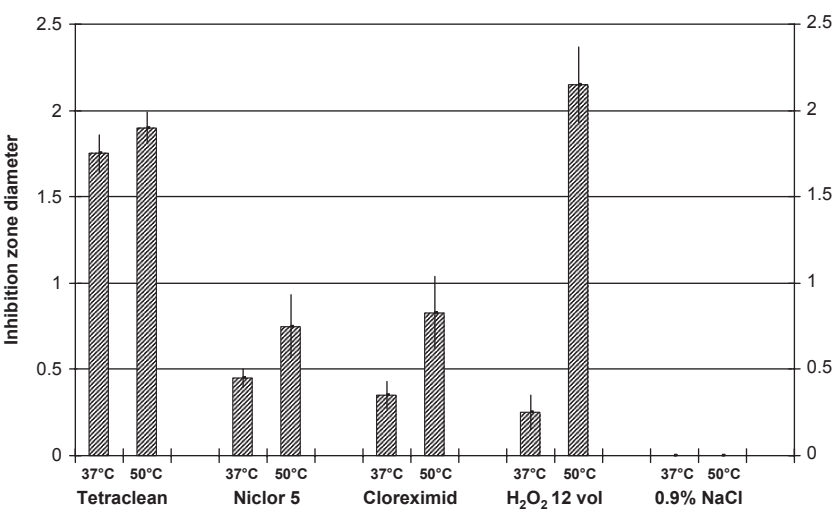

Fig. 2. Streptococcus mutans – inhibition zone diameter (mm) of different groups.

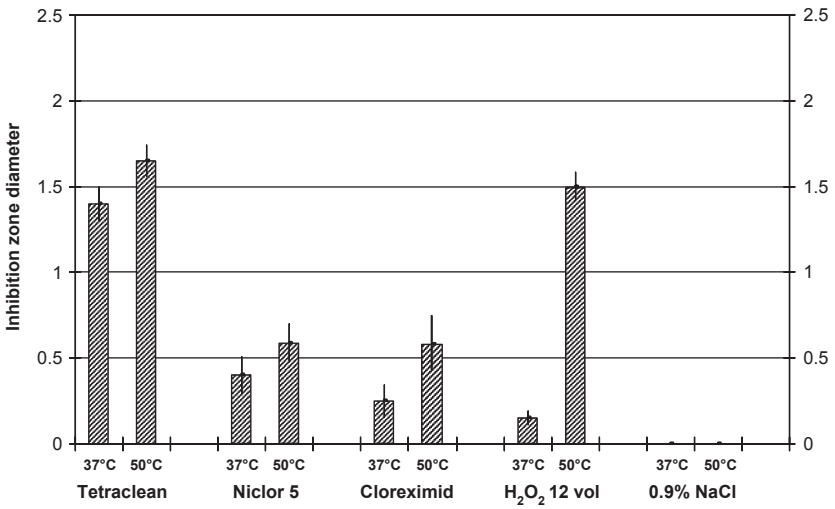

Fig. 3. Staphylococcus aureus – inhibition zone diameter (mm) of different groups.

when used as an endodontic irrigant. It has been proposed that hydrogen peroxide could be used in conjunction with sodium hypochlorite (5%) as an effective irrigation regimen. Ohara et al. (24) demonstrated a good germicidal ability of hydrogen peroxide. The results of this study confirmed that hydrogen peroxide 12 volumes is effective against the growth of all tested microorganisms. In fact, it showed the highest zones of inhibition. It was postulated that chlorhexidine solution might be an effective endodontic irrigant (24), because of its ability to be absorbed and released by dental tissues. In this way, it might act as a disinfectant of the tissues. Ayhan et al.(14) tested a solution of 2.0% chlorhexidine and reported its effective use as an endodontic irrigant. A 5.25% sodium hypochlorite has been recommended as an endodontic irrigant in the treatment of infected root canals, because of its well-known bactericidal action. The results of the agar disc-diffusion tests showed that 5.25% sodium hypochlorite was an effective agent against the growth of all the tested microorganisms. Preheating of sodium hypochlorite resulted in an even greater bactericidal effect. The bactericidal activity of sodium hypochlorite is because of the fact that when sodium hypochlorite is added to water, hypochlorous acid (HOCl), which contains active chlorine, a strong oxidizing agent, is formed. Substantial evidences suggest that chlorine exerts its antibacterial effect by the irreversible oxidation of –SH groups of essential enzymes, disrupting the metabolic functions of the bacterial cell (33). Pappen et al. (29) investigated the antibacterial effect of Tetraclean, MTAD and five experimental irrigants using both direct exposure test with planktonic cultures and mixed-species *in vitro* biofilm model: Tetraclean was more effective than MTAD against *E. faecalis* in planktonic culture and in mixed-species *in vitro* biofilm. The results from planktonic killing studies have to be interpretated with caution, and direct extrapolation to the agents' performance in complex *in vivo* systems is not possible. However, these tests may be useful for preliminary screenings of disinfecting agents before proceeding into more complex experimental designs (29). In the present study, the antibacterial activity of Tetraclean was most effective against the three microorganisms used, in agreement with Pappen et al. (29). For the pathogens tested, preheating at 50°C increases antibacterial activity of all tested endodontic irrigants. At 50°C preheated hydrogen peroxide 12 volumes and Tetraclean showed highest inhibition of the bacterial growth. At 37°C, Tetraclean showed the highest inhibition zones than all the other irrigants tested.

Acknowledgement

The authors deny any conflicts of interest.

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