

Antimicrobial effect of MTAD, Tetraclean, Cloreximid, and sodium hypochlorite on three common endodontic pathogens

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ABSTRACT

Objectives: The aim of this *in vitro* study was to evaluate the antimicrobial action of BioPure MTAD (Dentsply Tulsa Dental, Johnson City, TN), Tetraclean, Cloreximid (a mixture of Chlorhexidine (CHX) digluconate and Cetrimide), and 5.25% NaOCl (Ogna Laboratori Farmaceutici, Milano, Italy) against selected endodontic pathogens (*Enterococcus faecalis*, *Porphyromonas gingivalis*, and *Prevotella intermedia*).

Materials and Methods: The agar plate diffusion procedure was used to observe the antimicrobial activity of irrigants.

Results: Statistical analysis revealed significant effects of the different irrigants on the bacteria colonies. Treatment with 5.25% NaOCl induced a larger zone of microbial inhibition in *Prevotella intermedia* and *Porphyromonas gingivalis* (Tukey HSD post-test, $P = 0.0001$) when compared to MTAD, Tetraclean and CHX. Anyway, MTAD and Tetraclean were more effective to inhibit bacterial growth compared to CHX ($P < 0.0001$, Tukey HSD post-test). Furthermore, post hoc analysis revealed that MTAD and Tetraclean induced the largest zone of microbial inhibition of *Enterococcus faecalis* cultured under both aerobic and anaerobic conditions, when compared with 2% CHX and NaOCl ($P < 0.0001$, Tukey HSD post-test). The control group showed no microbial inhibition.

Conclusion: 5.25% NaOCl showed a high antimicrobial activity against anaerobic bacteria. MTAD and Tetraclean showed a high action against both, strictly anaerobic and facultative anaerobic bacteria. Chlorhexidine + Cetrimide (Cloreximid) showed the lowest antibacterial activity against both, facultative and strictly anaerobic bacteria tested.

Key words: MTAD, tetraclean, cloreximid, sodium hypochlorite, *Enterococcus faecalis*, *Porphyromonas gingivalis*, *Prevotella intermedia*

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Microorganisms are the main cause for pulp and periapical inflammation and disease. The failure to effectively eliminate them and their by-products might result in persistent irritation and impaired healing.^[1,2] A variety of irrigant solutions have been used in endodontics in order to eliminate or reduce the number of these bacteria. Irrigants are used during the endodontic treatment to flush out loose debris, to lubricate the dentinal walls, to dissolve organic matter in the canal, and to have antimicrobial effects.^[3] Different concentrations of sodium hypochlorite (NaOCl) have been used as root canal irrigants for the past seven decades because of its well-known antimicrobial action and its ability to dissolve tissues.^[4] NaOCl, however, has been proved cytotoxic, if introduced in periradicular tissues. If extruded, it may cause excruciating pain, immediate swelling, and profuse bleeding.^[5,6]

BioPure MTAD (Dentsply TulsaDental, Johnson City, TN) has been described as a universal irrigating

solution.^[7] Torabinejad *et al.*,^[8] have shown that MTAD is able to remove the smear layer and is effective against *Enterococcus faecalis*. Furthermore, it can eliminate bacteria in human root canals that had been infected by whole saliva.^[9] A new irrigant, Tetraclean (Ogna Laboratori Farmaceutici, Milano, Italy), a mixture of doxycycline hyclate—at a lower concentration than MTAD—with acid and detergents, has been developed. In a previous study, Tetraclean has shown the lowest value of surface tension, and this could help adapting this mixture to dentinal walls and to biofilm.^[10] In a biofilm model, Giardino *et al.*,^[11] have shown that Tetraclean was able to reduce 90% of bactericidal load after five minutes and >99.9% after 30 minutes of application. Chlorhexidine (CHX) has been recommended as an irrigating solution^[12] or intracanal dressing^[13,14] in endodontic therapy. Thanks to its properties, such as broad spectrum of antimicrobial activity, substantivity, low toxicity, and water solubility. CHX has gained much interest in endodontics. The aim of this *in vitro* study was to compare the antimicrobial action of Tetraclean, BioPure MTAD, Cloreximid [a mixture of CHX digluconate and Cetrimide (Ogna Laboratori Farmaceutici, Milano, Italy)],

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and 5.25% NaOCl against three bacterial species usually found in infected root canals.

MATERIALS AND METHODS

The microorganisms employed in this study were two obligate anaerobic bacteria (*Porphyromonas gingivalis* ATCC 33277 and *Prevotella intermedia* ATCC 25611), and one anaerobic facultative bacteria (*Enterococcus faecalis* ATCC 29212). These obligate anaerobic strains have grown in a 5-ml brain heart infusion broth (BHI) (Difco Co; Becton Dickinson, Sparks, MD), supplemented with hemin (5 mg/L) and menadione (0.5 mg/L) for seven days at 37°C. Facultative anaerobic strain (*Enterococcus faecalis* ATCC 29212) have grown in a 5-ml brain heart infusion broth for 24 hours at 37°C. Both cultures were then adjusted to 1 Mc Farland scale (3×10^8 CFU/ml). Each resulting suspension was spread with sterile swabs over the entire BHI supplemented with Vitamine K₁ and Hemin plates [Oxoid, Garbagnate M.se (Mi), Italy] surface of obligate anaerobes, and over the entire BHI agar of facultative anaerobe. The agar plate diffusion procedure was used to observe the antimicrobial activity of irrigants. After inoculation, five saturated paper disks, 6 mm in diameter, were placed on each agar plate. Four of them were saturated with one of the four test solutions, and one was saturated with sterile distilled water (control group). Sixteen BHI agar plates supplemented with Vitamine K₁ and Hemin (eight for *Porphyromonas gingivalis*, eight for *Prevotella intermedia*) were incubated for three days in sealed AnaeroJar with AnaeroGen sachet [OXOID, Garbagnate M.se (Mi), Italy] to create an anaerobic atmosphere prior to measuring the inhibition zones.^[15] OXOID Anaerobic Indicator (BR0055) was used to check whether the anaerobic conditions were achieved and maintained. Sixteen BHI agar plates of *Enterococcus faecalis* were randomly divided into two groups. Group one (n = 8) was incubated aerobically at 37°C for 48 hours. Group two (n = 8) was incubated anaerobically at 37°C for 72 hours. All assays were repeated three times to

ensure reproducibility. Microbial zones of inhibition were measured in millimeters. The data was statistically analyzed using the Statistica version 6 program (Statsoft, Tulsa, US). Repeated Measure Analysis of variance (ANOVA) followed by Tukey HSD post-test was applied, with a level of significance set at 5% ($P = 0.05$).

RESULTS

Statistical analysis showed remarkable effects of different irrigants on bacteria colonies [Treats \times F (12, 105) = 201, 88, $P = 0.0000$]. Treatment with water (control) [Figures 1-4] did not induce any microbial zones of inhibition either in both obligate anaerobic bacteria (*Porphyromonas gingivalis* ATCC 33277 or *Prevotella intermedia* ATCC 25611), or in anaerobic facultative bacteria (*Enterococcus faecalis* ATCC 29212). Treatment with 5.25% NaOCl induced a larger zone of microbial inhibition in *Prevotella intermedia* and *Porphyromonas gingivalis* (Tukey HSD post-test, $P = 0.0001$) [Figures 1 and 2] than MTAD, Tetraclean and Cloreximid. Anyway, MTAD and Tetraclean were more effective to inhibit bacterial growing than CHX ($P < 0.0001$, Tukey HSD post-test) [Figures 1 and 2]. Furthermore, post hoc analysis revealed that MTAD and Tetraclean induced the largest zone of microbial inhibition for both aerobic and anaerobic *Enterococcus faecalis* samples when compared with Cloreximid and 5.25% NaOCl ($P < 0.0001$, Tukey HSD post-test) [Figures 3 and 4]. The zones of inhibition with Cloreximid and 5.25% NaOCl were different from each other ($P < 0.0005$, Tukey HSD post-test) [Figures 3 and 4].

DISCUSSION

While preparing this study, the authors chose different bacterial strains present in different clinical patterns. *Enterococcus faecalis* is a Gram-positive, facultative anaerobic bacterium, present in postendodontic treatment

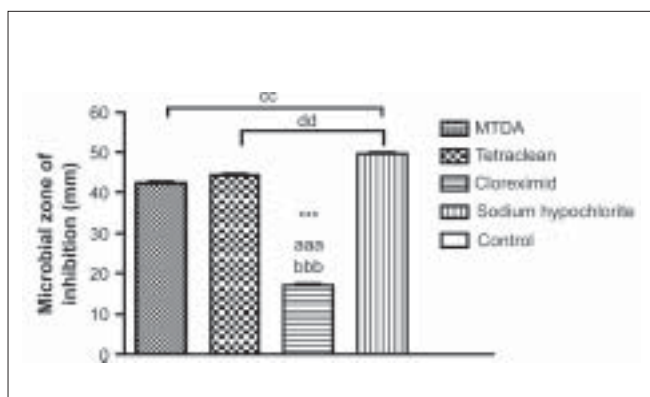


Figure 1: Zones of microbial inhibition induced by MTAD, Tetraclean, sodium hypochlorite, and Cloreximid in *Porphyromonas gingivalis* ATCC 33277 culture. Data are expressed as mean \pm SEM. *** $P < 0.0001$ Cloreximid vs. sodium hypochlorite, aaa $P < 0.0001$ Cloreximid vs. Tetraclean, bbb $P < 0.0001$ Cloreximid vs. MTAD; cc $P < 0.001$ Tukey's test post hoc multiple comparison

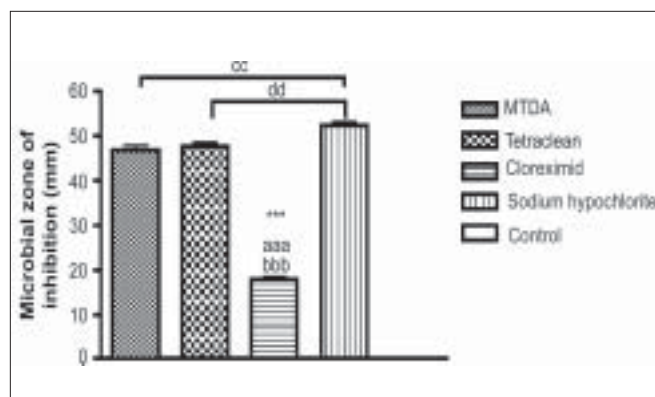


Figure 2: Zones of microbial inhibition induced by MTAD, Tetraclean, sodium hypochlorite, and Cloreximid in *Prevotella intermedia* ATCC 25611 culture. Data are expressed as mean \pm SEM. *** $P < 0.0001$ Cloreximid vs. NAOCL, aaa $P < 0.0001$ Cloreximid vs. Tetraclean, bbb $P < 0.0001$ CHX vs. MTAD; cc $P < 0.001$ Tukey's test post hoc multiple comparison

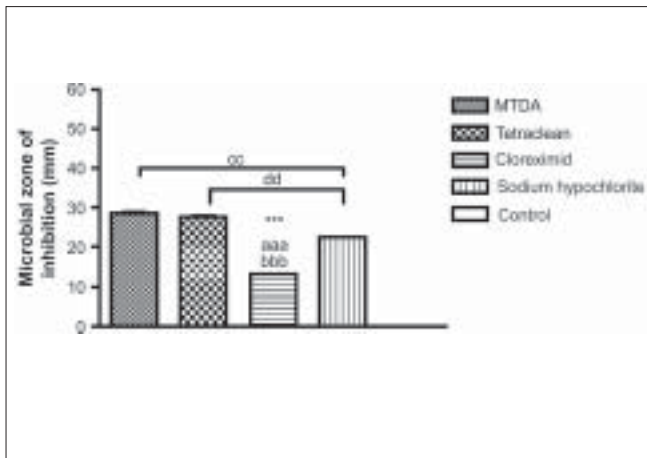


Figure 3: Zones of microbial inhibition induced by MTAD, Tetraclean, sodium hypochlorite, and Cloreximid in anaerobic facultative bacteria (*Enterococcus faecalis* ATCC 29212 culture (anaerobic condition)). Data are expressed as mean ± SEM. *** $P < 0.0001$ Cloreximid vs. sodium hypochlorite, aaa $P < 0.0001$ Cloreximid vs. Tetraclean, bbb $P < 0.0001$ Cloreximid vs MTAD; cc $P < 0.001$ Tukey's test post hoc multiple comparison

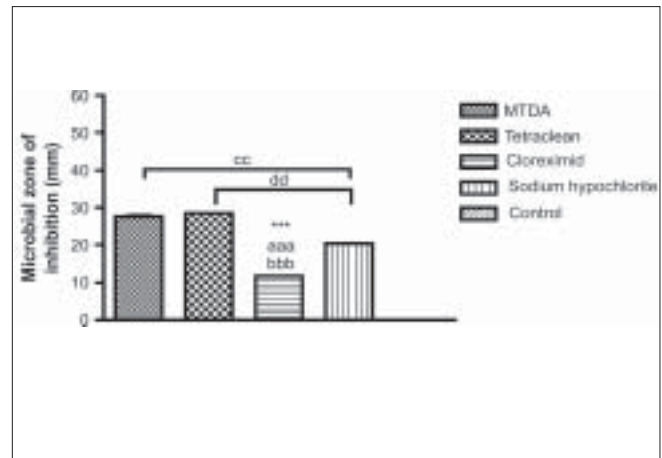


Figure 4: Zones of microbial inhibition induced by MTAD, Tetraclean, sodium hypochlorite, and Cloreximid in anaerobic facultative bacteria [*Enterococcus faecalis* ATCC 29212 culture (aerobic condition)]. Data are expressed as mean ± SEM. *** $P < 0.0001$ Cloreximid vs. sodium hypochlorite, aaa $P < 0.0001$ CHX vs. Tetraclean, bbb $P < 0.0001$ Cloreximid vs. MTAD; cc $P < 0.001$ Tukey's test post hoc multiple comparison

diseases.^[16] *Enterococcus faecalis* is seldom present in primary endodontic infections.^[17] *Porphyromonas spp.* (*gingivalis* and *endodontalis*) and *Prevotella spp.* (*intermedia* and *nigrescens*) were found oftene in primary infections (64% of teeth samples with necrotic pulp) than in secondary infections (36% of teeth with endodontic treatment failure).^[18] *Prevotella intermedia* and *Porphyromonas gingivalis* are two Gram-negative black pigmented anaerobic bacteria, isolated in acute apical periodontitis in primary endodontic infection. According to Gomes et al.,^[19] pain of endodontic origin is caused by *Prevotella spp.* (in association with *Peptostreptococcus spp.*), while Haapasalo^[20] found *Porphyromonas gingivalis* to be related to acute symptoms. Peters et al.,^[21] found *Prevotella intermedia* much oftene ($P < 0.05$) than all other species (except *Peptostreptococcus micros*) in endodontic samples taken from 58 roots of teeth with periapical bone distruction. *Porphyromonas gingivalis* is the most frequently isolated bacterium from root canals of infected teeth with periapical abscesses.^[22] Siqueira et al.,^[23] reported that Black Pigmented Bacteria are mainly found in cases of acute periradicular abscess. Most strains of *Porphyromonas spp.* and *Prevotella spp.* are not infective in monoculture: Their capability to induce periradicular lesion is probably due to a bacterial synergism that can influence the virulence of *Porphyromonas*, the most frequently detected species.^[24]

In this study, the authors used the “agar diffusion test” following the same procedures as Davis et al.^[25] The “agar diffusion test” is a common test used to evaluate the “in vitro” antimicrobial action of irrigants with no such variables as found in a tooth model. Some variations were made: BHI Agar plates supplemented with Hemin and K₁ Vitamin were used to grow *Porphyromonas gingivalis*

and *Prevotella intermedia*; strictly anaerobic bacteria (*Porphyromonas gingivalis* and *Prevotella intermedia*) and half of culture plates of facultative anaerobic bacteria were incubated for three days using AnaeroGen method.^[15]

In the present study, we tested the antibacterial activity of four irrigants against three bacteria, two of them present in primary infection and *Enterococcus faecalis*, known as the most important microorganism in post endodontic treatment disease. 5.25% NaOCl is the most common irrigant used in endodontics; Chloreximid is a mixture of Cetrimide (a surfactant agent) and Chlorexidine digluconate (0.2%), often used as an alternative irrigant. MTAD and Tetraclean are two mixtures of Doxycycline (a tetracycline isomer), Citric Acid and detergents. These irrigants differ in the kind of detergent and in antibiotic concentration (three times as much in MTAD).

The activity of tetracycline against *Porphyromonas gingivalis* was studied by Jacinto et al.^[22] In this study, the reaction of this Gram-negative rod to different antibiotics was tested. None of isolated strain was found resistant to tetracycline action. Carson et al.,^[26] reported that 0.01% Doxycyclin and 0.005% proved to be more effective on *Prevotella intermedia* than 6% NaOCl and 3% NaOCl. D'Arcangelo et al.,^[27] showed the complete elimination of *Enterococcus faecalis* and *Porphyromonas gingivalis* after 10' irrigation using both concentrations of NaOCl and Cetrimide + Chlorexidine. Ohara et al.,^[28] using the same methodology, reported that Chlorexidine displayed the most effective antimicrobial activity against the selected anaerobic bacteria. Chlorexidine was effective with *Porphyromonas gingivalis*, only after 1' even when diluted 400 times; 5.25% NaOCl was effective after 1' only

when diluted 10 times. In the present study all tested irrigants showed antimicrobial activity against *Prevotella intermedia* and *Porphyromonas gingivalis*. Nevertheless, important differences were found in the inhibition areas width, where Chlorhexidine + Cetrimide showed the lowest inhibition activity and 5.25% NaOCl the highest one. The general order of antimicrobial effectiveness was NaOCl > Tetraclean = MTAD > Cloreximid, but MTAD and Tetraclean were more effective to inhibit bacterial growth than Cloreximid ($P < 0.0001$). Examining the action of the tested irrigants on *Enterococcus faecalis*, the general order of antimicrobial effectiveness was Tetraclean = MTAD > 5.25% NaOCl > Cloreximid. Antibiotic-based irrigants induced the largest zone of microbial inhibition for both aerobic and anaerobic *Enterococcus faecalis* samples as compared to Cloreximid and 5.25% NaOCl ($P < 0.0001$). Contrary to Davis *et al.*,^[25] the inhibition zones with Cloreximid and 5.25% NaOCl were different from each other ($P < 0.0005$).

In an “*in vitro*” study, using the “agar diffusion test”, Torabinejad^[29] compared the action of MTAD and 5.25% NaOCl. This study showed that, when undiluted, MTAD and 5.25% NaOCl have the same antimicrobial activity (35 mm and 34 mm respectively). In the present study, the inhibition areas were narrower and there were statistically remarkable differences in the action of the two irrigants. This last datum is in compliance with Davis *et al.*^[25] MTAD and Tetraclean are very similar, containing the same antibiotic (doxycycline), citric acid and a detergent. Although the doxycycline concentration was three times as high, in the present study the inhibition area of these irrigants was the same. According to Davis, in the present study no differences in antimicrobial action of tested irrigants were present when samples were either anaerobically or aerobically incubated. In this study, Chlorhexidine + Cetrimide showed the worst action against all tested bacteria. As underlined by Davis *et al.*,^[25] this kind of test “does not address the property of substantivity of the medicament, only the ability to inhibit the growth of microorganism”. Therefore, Cloreximid may still clinically have a longer antimicrobial effect on *Enterococcus faecalis* than the other tested irrigants. Portenier *et al.*,^[30] however, showed that the action of MTAD and 2% Chlorhexidine was inhibited by the dentin powder. Further clinical studies should be therefore performed to determine the real “*in vivo*” action of antibiotic-based irrigants on *Enterococcus faecalis*.

CONCLUSION

In this study, 5.25% NaOCl showed a high antimicrobial activity against anaerobic bacteria responsible for primary endodontic infection, but its action seems to be much lower against *Enterococcus faecalis*. MTAD and Tetraclean showed a high action against both, strictly anaerobic and facultative

anaerobic bacteria. Chlorhexidine + Cetrimide (Cloreximid) showed the lowest antibacterial activity against both, facultative and strictly anaerobic bacteria tested.

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