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
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. Faculative 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 × 10⁸ 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 × 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 samples when compared with Cloreximid and 5.25% NaOCl (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...
Enterococcus faecalis is seldom present in primary endodontic infections. Porphyromonas spp. (gingivalis and endodontalis) and Prevotella spp. (intermedia and nigrescens) were found oftene in primary infections (64% of teeth with necrotic pulp) than in secondary infections (36% of teeth with endodontic treatment failure). 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., pain of endodontic origin is caused by Prevotella ssp. (in association with Peptostreptococcus ssp.), while Haapasalo found Porphyromonas gingivalis to be related to acute symptoms. Peters et al. 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 destruction. Porphyromonas gingivalis is the most frequently isolated bacterium from root canals of infected teeth with periapical abscesses. Siqueira et al. 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 periapical lesion is probably due to a bacterial synergism that can influence the virulence of Porphyromonas, the most frequently detected species.

In this study, the authors used the “agar diffusion test” following the same procedures as Davis et al. 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.

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 Doxicycline (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. 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. 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. showed the complete elimination of Enterococcus faecalis and Porphyromonas gingivalis after 10’ irrigation using both concentrations of NaOCl and Cetrimide + Chlorexidine. Ohara et al. 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 Chlorexidine + Cetrime showed the lowest inhibition activity and 5.25% NaOCl the highest one. The general order of antimicrobial effectiveness was NaOCl > Tetraclean = MTAD > Cloreximid, and 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, Chlorexidine + Cetrime 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% Chlorexidine 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. Chlorexidine + Cetrime (Cloreximid) showed the lowest antibacterial activity against both, facultative and strictly anaerobic bacteria tested.

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