Comparison of the Antimicrobial Activity of Six Irrigants on Primary Endodontic Pathogens

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Abstract

The purpose of this study was to compare the antimicrobial activities of 6% and 3% sodium hypochlorite (NaOCl), 2% and 0.12% chlorhexidine gluconate (CHX), and 0.01% and 0.005% doxycycline (Doxy) on four microorganisms associated with primary endodontic infections. The agar diffusion test was used to measure antimicrobial activities of these agents against *Peptostreptococcus micros*, *Prevotella intermedia*, *Streptococcus sanguis*, and *Lactobacillus acidophilus.* Minimum inhibitory concentration analysis was performed using the macrodilution method. For three of the four microorganisms, the general order of antimicrobial effectiveness was 0.01% Doxy >0.005% Doxy >6% NaOCl >3% NaOCl >2% CHX > 0.12% CHX. For *L. acidophilus*, the order of effectiveness was 6% NaOCl >3% NaOCl >2% CHX > 0.01% Doxy >0.005% Doxy >0.12% CHX. The 6% NaOCl showed significantly greater zones of inhibition than 3% NaOCl for all endopathogens tested.

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Recently, much attention has been focused in the literature regarding antimicrobial regimens against pathogens associated with endodontic retreatment (1, 2). However, the great majority of endodontic disease is primary in nature. The role of bacteria in the development in apical periodontitis has been well established (3). A study by Moller et al. found that those teeth with infected pulp tissue induced inflammatory reactions in the periradicular tissues. Numerous studies have shown that the bacterial flora in endodontic infections is polymicrobial with a predominance of anaerobic species (4, 5). Using advanced anaerobic bacteriological techniques, Lana et al. showed a polymicrobial environment in necrotic teeth that consisted of obligate and facultative anaerobes, microaerophilic bacteria and yeast (6). Eradication of these microorganisms in the root canal space is paramount in preventing them from reaching the periapical tissues.

With such a complex and dynamic microbial environment in the root canal system, selection of an effective antibacterial agent to use during treatment is critical. Antimicrobial solutions must possess many qualities such as the ability to penetrate the infected site, to suppress or destroy microbial growth, and to avoid the possible development of resistance to the agent (7). Tissue remnants in the canal may provide enough nutrients for bacterial survival. Irrigating solutions also possessing the ability to dissolve organic material are desirable in endodontic treatment.

Sodium hypochlorite (NaOCl) is a commonly used irrigating solution that has been shown to have both antimicrobial and tissue dissolving properties (8, 9). However, there is concern about its possible toxic effect on the periapical tissues at higher concentrations. At lower concentrations, however, not only is its tissue dissolving ability reduced, but its antimicrobial effectiveness as well (10, 11). An alternative irrigant that has been proposed is chlorhexidine gluconate (CHX), studied primarily because of its substantivity (12). A 5.25% concentration of NaOCl was shown to be no more toxic than less concentrated NaOCl or 0.12% CHX when injected into the subcutaneous tissue of a guinea pig (13).

Jeansonne et al. found no significant difference between 5.25% NaOCl and 2% CHX in colony forming units when testing growth media aspirates from extracted teeth (14). However, Siqueira et al. found 4% NaOCl to be significantly more antibacterial than 2% CHX in an agar diffusion model (15). Recently, an experimental disinfecting agent (MTAD) containing a mixture of a tetracycline isomer (doxycycline), an acid (citric acid), and a detergent (Tween 80) was shown to be a superior antimicrobial agent to 5.25% NaOCl in an agar diffusion model (16). No study to date has compared the in vitro antimicrobial activities of NaOCl, CHX, and doxycycline (Doxy).

The purpose of this study was to compare the antimicrobial activities of 6% and 3% sodium hypochlorite, 2.0% and 0.12% chlorhexidine gluconate, and 0.01% and 0.005% doxycycline on four microorganisms commonly associated with primary endodontic infections.

Methods and Materials

The freeze-dried microorganisms studied in this experiment were the following: *Peptostreptococcus micros* (ATCC #33270), *Prevotella intermedia* (ATCC #25611), *Streptococcus sanguis* (ATCC #10556), and *Lactobacillus acidophilus* (ATCC #11975). The cultures were perpetuated on a weekly basis. The obligate anaerobes were cultured on plates containing Brucella agar with 5% sheep blood, hemin, and vitamin K (Remel Co., # 04012). *S. sanguis* growth was maintained on chocolate media (Remel Co., #01300). Lactobacillus agar plates were fabricated as follows. In a 1000 ml

beaker of sterile water (Braune Medical, Irvine, CA), 75 mg of Rogosa agar powder (Difco Co., Becton Dickinson, Sparks, MD) was added to 5% glacial acetic acid (Fisher Scientific Co., Middletown, VA). After reaching a boil, the mixture was cooled and the plates were made.

After 48 h growth of each microorganism, a nephelometer (CrystalSpec, Sparks, MD), was used to obtain a 0.5 McFarland standard. This standard is equivalent to 1.5×10^8/ml cell density. The six irrigants introduced to the plates were 6% and 3% solutions of NaOCl (Ultra-Clorox, Oakland, CA), 2.0% CHX prepared by dilution of a lab grade 20% preparation (Sigma-Aldrich Co., St. Louis, MO), 0.12% CHX (Alpharma USPD Inc., Baltimore, MD), 0.01% Doxy prepared from a 1:2 $\times$ 12 dilution of a 100 mg/ml lyophilized intravenous solution (Doxy 100, American Pharmaceutical Partners, Los Angeles, CA) and 0.005% Doxy prepared from a 1:2 $\times$ 13 dilution of the above solution.

For each microorganism, sixteen plates were inoculated using a three-way streak. For testing NaOCl and CHX solutions, seven plates were divided into five sections; four sections to receive the test agents and one site to receive a disk with sterile water (Braune Medical, Irvine, CA) that served as a negative control. For testing doxycycline solutions, seven plates were divided into three sections; two sites to receive the Doxy agents and one site to receive a disk with sterile water that served as a negative control. One inoculated plate in each group was left open to the air during processing to insure no contaminants were introduced.

There were 6-mm sterile paper disks were then saturated with the test solutions and transferred to the designated sections on the inoculated plates. The obligate anaerobes were placed in an anaerobic chamber containing 3 Pack-Anaeros (Remel Co.) and an anaerobic indicator (Remel Co.). All plates were then placed in an incubator at 37°C for 48 to 72 h. The resulting zones of inhibition were measured by an independent observer using a digital caliper (L.S. Starrett Co., Athol, MA) with a sensitivity of ± 0.02 mm.

Statistical analysis was performed using a one-way ANOVA for the mean zones of inhibition among the materials tested ($p < 0.05$). The Student Newman-Keuls test was run for multiple comparisons.

All experimental irrigants were serially diluted 1:1 five times with sterile phosphate buffered saline (PBS) (ICN Biomedicals, Inc., Aurora, OH) for minimum inhibitory concentration (MIC) analysis. A 0.5 McFarland equivalent was created for each organism in Schaelder broth (Lenexa, KS). One milliliter of each microorganism solution was transferred to test tubes containing 1 ml of each of the original and diluted test irrigants. This resulted in up to a 64x dilution for each irrigant. All tubes were sealed tightly and incubated for 48 to 72 h. The turbidity was measured using the nephelometer to indicate bacterial inhibition.

Results

The results showed that both the 0.01% and 0.005% solutions of doxycycline had significantly greater zones of inhibition than 6% NaOCl for *P. micros*, *P. intermedia*, and *S. sanguis*. 6% NaOCl was significantly more effective at inhibiting growth of *L. acidophilus* when compared to all other irrigants. In addition, 6% NaOCl had significantly greater zones of inhibition than 3% NaOCl for all organisms tested. The 0.12% CHX showed significantly smaller zones of inhibition for all endopathogens compared to all other irrigants. The order of antibacterial effectiveness is listed in Table 1.

Concerning minimum inhibitory concentration analysis, no samples showed turbidity as measured by the nephelometer after 48 and 72 h. All controls displayed turbidity. In accordance with National Naval Medical Center Infectious Disease Department reporting procedure, the minimum inhibitory concentrations for all organisms tested were below the following levels: sodium hypochlorite, 469 mcg/ml; chlorhexidine gluconate, 19 mcg/ml; and doxycycline, 0.78 mcg/ml.

Discussion

The selection of test endopathogens for this study was arbitrary and not a random sampling from necrotic teeth. Therefore, the results should not be automatically extrapolated globally. Nevertheless, an attempt was made to select representative Gram negative/positive and anaerobic/aerobic bacteria that have been commonly isolated from necrotic canals.

Doxycycline was chosen for this experiment because it is one of the components of MTAD. Pilot studies showed that a significant dilution of the Doxy 100 solution was needed to keep zones of inhibition inside the edges of the agar plates. Therefore, greater inhibition for the organisms with this antibiotic was expected. The more interesting findings of the study were the relationships among the other irrigants for each organism.

This study showed that 6% NaOCl had significantly more antimicrobial activity than 3% NaOCl for all organisms tested on three media. These results were consistent with those found in a recent study by Vianna et al. in which a reduction in concentration of 5.25% NaOCl decreased antimicrobial effectiveness against the anaerobes tested (17). When considering chlorhexidine gluconate as a primary irrigant, some studies have shown 2% CHX to have more antimicrobial activity than 5.25% NaOCl (14, 18). However, this agar diffusion study showed 6% NaOCl to be more effective than 2% CHX. The differences in these results may be a result of the different diffusion coefficients of the agents. The relevance of the agar diffusion model to the in vivo situation remains unclear.

In any case, antimicrobial activity is not the only requirement for an endodontic irrigant (19). Despite their antibacterial properties, neither chlorhexidine nor doxycycline has been shown to dissolve tissue in the root canal system. In a recent study, 2% CHX gel and liquid solution showed no tissue dissolution capabilities during the observed 6 h of measurement (20). Therefore, chlorhexidine does not possess a main property of an ideal primary endodontic irrigant.

Five percent of sodium hypochlorite has been shown to dissolve necrotic pulpal tissue faster than 2.5% NaOCl or lower concentrations (21). In addition, other studies have shown a decrease in the tissue dissolving capability of NaOCl with a decrease in concentration (11, 22). To obtain maximum benefit in necrotic tissue dissolution, full strength NaOCl would be ideal.

Serious concerns exist about the advisability of routinely using intracanal antibiotics in endodontic treatment. With the possible leakage of irrigating agents out apical or accessory foramina, the potential

TABLE 1. Order of antimicrobial effectiveness

Test organism	Order of effectiveness/significance
Peptostreptococcus micros	.01% Doxy > .005% Doxy > 6% NaOCl > 3% NaOCl > 2% CHX > .12% CHX
Prevotella intermedia	.01% Doxy ~ .005% Doxy > 6% NaOCl ~ 2% CHX > 3% NaOCl > .12% CHX
Streptococcus sanguis	.01% Doxy > 6% NaOCl ~ .005% Doxy > 3% NaOCl > 2% CHX > .12% CHX
Lactobacillus acidophilus	6% NaOCl > 3% NaOCl > 2% CHX ~ .01% Doxy ~ .005% Doxy > .12% CHX

> indicates significant difference at $p < .05$, ~ indicates no significant difference.

exists for hypersensitivity reactions or the development of bacterial antibiotic resistance. Although Torabinejad et al. found MTAD to be more antimicrobial than 5.25% NaOCl (16), the ability of MTAD to dissolve pulp tissue is not comparable to 5.25% NaOCl (23). In addition, although MTAD has been shown relatively effective at removing the smear layer (24), numerous studies have shown the superior ability of the combination of 17% ethylene diamine tetra-acetic acid (EDTA) and 5.25% NaOCl in this regard (25).

A possible irrigant for a final rinse may be chlorhexidine gluconate. It possesses the ability to sustain antimicrobial activity up to 21 days (26), which may be the time needed to kill those bacteria penetrating 382 microns in the tubules (27). It has also been shown to possess substantivity similar to doxycycline (28), with the added benefit of avoiding possible negative biological sequela of antibiotics. In a susceptibility testing study that included tetracycline, 17% of the Prevotella species were shown to be resistant to the antibiotics tested (29). This study also found that some of the Prevotella species carried the resistant gene for tetracycline known as tetQ.

Because of worldwide concern about the overuse and development of resistance to antibiotics (30, 31), the best option for a primary endodontic irrigant may be 6% NaOCl. Full strength is recommended over lower concentrations because of its superior antimicrobial and tissue dissolution properties. Because of the same concern, irrigation with a 2% CHX solution may be safer than an antibiotic. After smear layer removal with 17% EDTA and 6% NaOCl, 2% CHX is suggested as a final antimicrobial rinse for canal disinfection.

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