

Evaluation of the Antifungal Substantivity of Sodium Hypochlorite, Chlorhexidine and MTAD

Z Mohammadi

Citation

Z Mohammadi. *Evaluation of the Antifungal Substantivity of Sodium Hypochlorite, Chlorhexidine and MTAD*. The Internet Journal of Dental Science. 2006 Volume 5 Number 2.

Abstract

The purpose of this in vitro study was to compare the antifungal substantivity of 1% MTAD, 2% Chlorhexidine (CHX) and 1.3% sodium hypochlorite (NaOCl) in bovine root dentin. One hundred and ten dentin tubes prepared from bovine incisors were infected in vitro for 72 hours with *Candida albicans*. The specimens were divided into five groups as follows: CHX; MTAD; and NaOCl; infected dentin tubes (positive control); and sterile dentin tubes (negative control). Dentin chips were collected with round burs into tryptic soy broth (TSB). After culturing the number of colony-forming units (CFU) was counted. In all experimental groups CFU was minimum in the first cultures, and the results obtained were significantly different from each other at any time period ($P < 0.05$). In first culture, the NaOCl group and CHX group showed the lowest and highest number of CFU, respectively. In each group, the number of CFUs increased significantly by time-lapse ($P < 0.05$). In conclusion, the substantivity of MTAD was significantly greater than CHX and NaOCl.

INTRODUCTION

Studies have shown that endodontic infections are polymicrobial with a predominance of anaerobic species (1). Throughout the past decades, it has been well known that yeasts can be isolated from infected root canals. Fungi were observed both in primary and refractory endodontic infections. Among those fungal infections, *C. albicans* was the most frequently found type. The number of yeast cells in the root canal is usually lower than that of bacteria. It has been hypothesized that if there is a yeast infection in the root canal, the use of common antifungal solutions during endodontic therapy may favor the overgrowth of yeasts between visits.

NaOCl is the most commonly used root canal irrigant, which has both antimicrobial and tissue dissolving properties (4, 5). *C. albicans* has proved very susceptible to its action in as little as 0.5% concentration within 10 seconds of contact time.

CHX (Sigma Chemicals Co., St. Louis, MO, USA), seems to act by adsorbing onto the cell wall of the microorganism and causing the leakage of intracellular components (6). Antimicrobial substantivity of CHX in the root canal system has been reported from 48 h to 21 days (7,8,9). However, Rosenthal et al. (1) indicated that substantivity of CHX was extended for up to 12 weeks.

MTAD (Dentsply Tulsa Dental, Tulsa, USA), a mixture of doxycycline, citric acid and a detergent (Tween 80), has recently been introduced as a final irrigant for disinfection of the root canal system. Shabahang et al. showed that MTAD was a more effective disinfectant of the root canal system than 5.25% NaOCl (10). They also found that the combination of 1.3% NaOCl and MTAD as a final treatment eliminated *E. faecalis* from human tooth cementum and dentin (11, 12). Ruff et al. (13) evaluated the antifungal efficacy of 6% NaOCl, 2% CHX, 17% EDTA and BioPure MTAD as a final rinse on *C. albicans* in vitro. They found that 6% NaOCl and 2% CHX were equally effective and statistically significantly superior to MTAD and 17% EDTA.

However, its antifungal substantivity has not been yet evaluated. The purpose of this study was to compare the antifungal substantivity of MTAD, CHX, and doxycycline against *E. faecalis* in bovine root dentin in vitro.

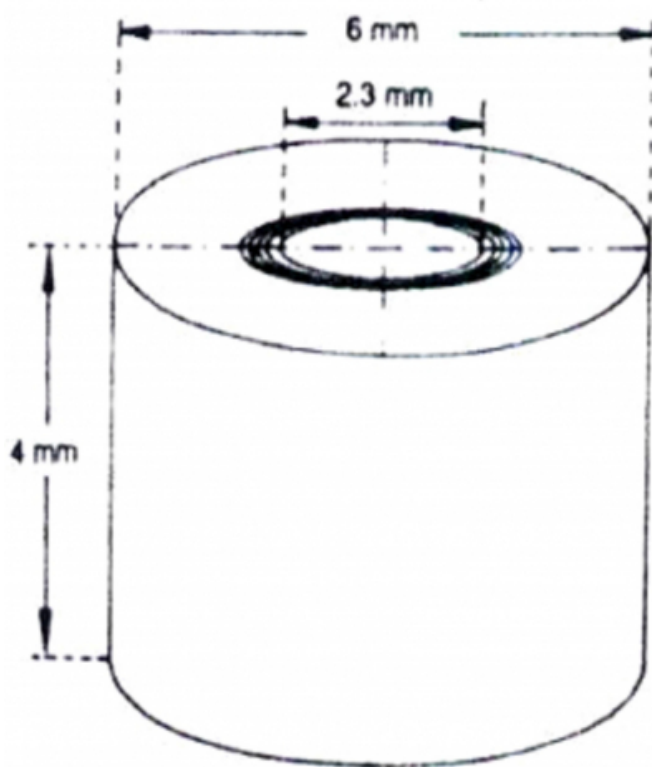
MATERIALS AND METHODS

The method used was a modification of the one previously described by Haapasalo and Orstavik (3). Forty intact bovine central incisors were selected for this study. The specimens were kept in 0.5% NaOCl solution for no longer than seven days. The apical 5 mm and two-thirds of the crown were removed from each tooth with a rotary diamond saw at 1000 rpm (Isomet Plus precision saw, Buehler, IL) under water-

cooling. Cementum was removed by using polish paper (Ecomet 3, variable-speed grinder-polisher, Buehler, IL), which resulted in a center-holed piece of root dentin with 6 mm outer diameter (Fig. 1). The roots were then cut into 4-mm thick slices with a diamond saw as above. The canals of the 4-mm blocks were enlarged with an ISO 023 round bur using slow speed. All teeth and dentin slices were preserved in vials containing tap water during the procedures to avoid dehydration. The dentin tubes (n=110) were individually treated with 5.25% NaOCl, and 17% EDTA (with pH 7.2) to remove the smear layer. The specimens were then placed in BHI broth (Oxoid, Basingstoke, UK) and autoclaved. They were then kept in an incubator at 37° C for 24 hours to check the efficacy of the sterilization.

Figure 1

Figure1: Schematic view of used dentin tubes (adopted from Gomes et al. ())



A total of 110 specimens were randomly divided into five groups as follow: Group 1 (30 specimens): MTAD, Group 2 (30 specimens): 2% CHX, Group 3 (30 specimens): 2.6% NaOCl, Group 4 (10 specimens): positive control (infected dentin tubes), Group 5 (10 specimens): negative control (sterile dentin tubes).

A suspension of *C. albicans* (ATCC 60193) was adjusted to 0.5 turbidity on the McFarland scale (1.5×10^8). All

specimens except for the negative controls were placed in plastic vials and 2 ml of adjusted *C. albicans* suspension was injected into the tubes to submerge the specimens. Negative controls were submerged in sterile saline. The samples were incubated at 36 C and 91% humidity for 72 hours. Every 24 hours the vial containing each sample was replenished with freshly made suspension of *C. albicans*. At 48 hours 1 µl aliquots was taken from each tooth using a calibrated inoculation loop (C.C.P. Scientific, Inc., Poetone, IL) and plated on Sabouraud 4% dextrose agar plates to verify the growth of *C. albicans* in each sample tube. Following the contamination period, each specimen was removed from its bottle under aseptic conditions and the canal irrigated with 5 ml of sterile saline and dried with sterile paper points. The outer surface of each specimen was covered with two layers of nail varnish, in order to prevent contact of the medicament with the external surface. Then, specimens were fixed at the bottom of wells of 24 -well cell culture plates with decontaminated sticky wax, which also obliterated the apical surface of the root canal. Finally, the irrigation solutions were applied to the canal lumen with sterile 3ml plastic syringes and 27- gauge needles until the dentin tubes were totally filled. Five minutes after placement of irrigants, solutions were removed using sterile paper points. Then, specimens were incubated at 37° C for a period of 28 days to maintain humidity. Dentin chips were removed from the canals with sequential sterile low - speed round burs with increasing diameters of ISO sizes: 025, 027, 029, 031, and 033 at experimental times of 0,7,14,21, and 28 days. Each bur removed approximately 0.1 mm of dentin around the canal. The powder dentin samples obtained with each bur were immediately collected in separate test tubes containing 3 ml of freshly prepared fluid thioglycolate broth. Thereafter, 100 microlitres from each test tube was cultured on Sabouraud dextrose agar (selective media). Growing colonies were counted and recorded as CFU. Results were analysed using analysis of variance and covariance with repeated measures (ANOVA) to indicate differences between the experimental groups and the positive control. One-way ANOVA (Tukey's method) was used to indicate differences within each layer.

RESULTS

The CFUs represent a close estimate of the number of viable fungi that penetrated into the dentinal tubules at different layer depths. The number of CFU obtained from five consecutive dentinal layers is presented in Table 1. The number of CFU in all three experimental groups was minimum in the first cultures. The positive control group

showed viable fungi at all experimental times, which indicated the efficiency of the method. In contrast, negative control group showed no viable fungi at all experimental times. At Day 0, the NaOCl group demonstrated the most effective antifungal action. However, at days 7, 14, 21, and 28, MTAD demonstrated the most effective antifungal action ($p < 0.05$).

Figure 2

Table 1: Means of the CFU and the Standard Deviation of *C. albicans* in experimental groups

	Day 0	Day 7	Day 14	Day 21	Day 28
NaOCl	0.50±0.68	16.11±8.04	38.40±8.99	63.80±11.12	87.55±5.50
CHX	4.72±3.62	11.10±3.73	17.43±4.54	39.30±5.33	59.66±5.33
MTAD	0.80±3.95	5.46±2.32	8.70±2.76	19.20±3.22	42.44±5.55

DISCUSSION

Current techniques of debridement leave many areas of the root canal completely untouched by the instruments (¹⁴). Thus, a root canal irrigant is needed to aid in the debridement of the canals. *C. albicans* was chosen for inoculum in this study because it is resistant to intracanal medication with calcium hydroxide (⁶) and plays an important role in the second endodontic infections. Residual antifungal activity of MTAD had not been yet evaluated. In the present study, the antifungal substantivity of NaOCl, CHX, and MTAD was monitored for 28 days. The 2.6% NaOCl solution had the most effective antifungal action at the first culture, but its antifungal action dropped rapidly.

This indicates that NaOCl has little to no antifungal substantivity. The probable reason is that NaOCl has an antimicrobial effect as long as free chlorine is available in the solution. The mean numbers of CFU were statistically lower in MTAD compared to other solutions at all experimental periods except for the first time, thus stressing the ability of MTAD to adsorb to hydroxyapatite with prolonged gradual release at therapeutic levels. Ruff et al. (¹³) evaluated the antifungal efficacy of 6% NaOCl, 2% CHX, 17% EDTA, and BioPure MTAD as a final rinse on *C. albicans* in vitro. They found that 6% NaOCl and 2% CHX were equally effective and statistically significantly superior to MTAD and 17% EDTA.

Shabahang and Torabinejad (¹²) compared the antibacterial effect of MTAD with that of NaOCl with and without EDTA. Their findings showed that the combination of 1.3% NaOCl as a root canal irrigant and MTAD as a final rinse

was significantly more effective than the other regimens. Kho and Baumgartner (¹⁵) compared the antimicrobial efficacy of irrigating with 1.3% NaOCl/ MTAD versus irrigation with 5.25% NaOCl/15% EDTA in the apical 5 mm of roots infected with *E. faecalis*. Their results demonstrated that there was no difference in antimicrobial efficacy for irrigation with 5.25% NaOCl/15% EDTA versus irrigation with 1.3% NaOCl/ MTAD. In another study Krause et al. (¹⁶) compared the antibacterial effect of MTAD, NaOCl, doxycycline, and citric acid on *E. faecalis*. Their findings showed that NaOCl was more effective than other solutions. Khademi et al. (¹⁷) compared the antibacterial substantivity of 2% CHX, 100 mg /ml doxycycline, and 2.6% NaOCl in bovine root dentin in vitro. They found that substantivity of CHX was significantly greater than doxycycline, and NaOCl, which is in contrast to the findings of the present study. It seems that the presence of a detergent (Tween 80) in MTAD increases the depth of penetration of this material into dentinal tubules by decreasing surface tension.

Rosenthal et al (¹). found that that treatment with a 2% solution of CHX induced substantivity for up to 12 weeks, which is in contrast to the findings of the present study. However, White et al. (⁷) concluded that antimicrobial activity of 2% CHX as a canal irrigant lasted 72 hours. In an in vivo study to evaluate the substantivity of 2% CHX as root canal irrigating solution, Leonardo et al. (⁸) found that CHX prevents microbial activity with residual effects in the root canal system up to 48h, whereas the present study showed that substantivity of 2% CHX was remained for 28 days. Komorowski et al. (¹⁷) reported that for induction of substantivity, dentin should be treated with CHX for 7 days and 5 min. treatment with CHX did not induce substantivity, which is in contrast to the findings of the present study.

Lin et al. (¹⁸) attributed the limited antifungal effect of CHX irrigation to absorb the medication to dentin during the first hour and stated that only after the saturation point after the first hour that the antifungal capability of CHX increase with time. Stabholz et al. (¹³) found that the antimicrobial substantivity of CHX was significantly lesser than tetracycline HCl 50 mg/ml for 12 days.

CONCLUSION

Under the conditions of the present study, the antifungal substantivity of MTAD was significantly higher than CHX and retained in root canal dentin for at least 28 days. Furthermore, NaOCl displayed very low substantivity.

References

1. Rosenthal S, Spangberg L, Safavi K. Chlorhexidine substantivity in root canal dentin. *Oral Surg Oral Med Oral Pathol* 98: 488-492, 2004.
2. Trope M, Bergenholz G. Microbiological basis for endodontic treatment: can a maximal outcome be achieved in one visit? *Endod Topics* 1: 40-53, 2002.
3. Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. *J Dent Res* 66: 1375-1379, 1987.
4. Bystrom A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. *Oral Surg Oral Med Oral Pathol* 55: 307-312, 1983.
5. Hasselgren G, Olsson B, Cvek M. Effect of calcium hydroxide and sodium hypochlorite and dissolution of necrotic porcine muscle tissue. *J Endod* 14: 125 -127, 1998.
6. Gomes BP, Souza SFC, Ferraz CCR, et al. Effectiveness of 2% Chlorhexidine gel and calcium hydroxide against *Enterococcus faecalis* in bovine root dentin in vitro. *Int Endod J* 36: 267-275, 2003.
7. White RR, Hays GL, Janer LR. Residual antimicrobial activity after canal irrigation with Chlorhexidine. *J Endod* 23: 229 -231, 1997.
8. Leonardo MR, Tanomaru Filho M, Silva LAB, Nelson Filho P, Bonifacio KC, Ito IY. In vivo antimicrobial activity of 2% Chlorhexidine used as a root canal irrigating solution. *J Endod* 25: 167-171, 1999.
9. Basrani B, Santos JM, Tjaderhane L, et al. Substantive antimicrobial activity in Chlorhexidine treated human root dentin. *Oral Surg Oral Med Oral Pathol* 94: 240 - 242, 2002.
10. Torabinejad M, Khademi AA, Babagoli J, et al. A new solution for the removal of the smear layer. *J Endod* 29: 170-175, 2003.
11. Torabinejad M, Shabahang S, Apicco R, Kettering JD. The antimicrobial effect of MTAD: An in vitro investigation. *J Endod* 29: 400-3, 2003.
12. Shabahang S, Torabinejad M. Effect of MTAD on *Enterococcus faecalis*- contaminated root canals of extracted human teeth. *J Endod* 29: 576-9, 2003.
13. Ruff ML, McClanahan SB, Babel BS. In vitro antifungal efficacy of four irrigants as a final rinse. *J Endod* 2006; 32:331-3.
14. Stabholz A, Kettering JD, Apicco R, Zimmerman G, Baker PJ, Wikesjo UM. Retention of antimicrobial activity by human root surfaces after in situ subgingival irrigation with tetracycline HCL or Chlorhexidine. *J Periodontol* 64:137-141, 1993.
15. Shabahang S, Pouresmail M, Torabinejad M. In vitro antifungal efficacy of MTAD and sodium hypochlorite. *J Endod* 29: 450-52, 2003.
16. Kho P, Baumgartner JC. A comparison of the antimicrobial efficacy of NaOCl/Biopure MTAD versus NaOCl/EDTA against *Enterococcus faecalis*. *J Endod* 32: 652-5, 2006.
17. Krause TA, Liewehr FR, Hahn CL. The antimicrobial effect of MTAD, sodium hypochlorite, doxycycline, and citric acid on *Enterococcus faecalis*. *J Endod* 33; 28-30, 2007.
18. Khademi AA, Mohammadi Z, Havaee A. Evaluation of the antifungal substantivity of several intra-canal agents. *Aust Endod J* 32:112-115, 2006.
19. Komorowski R, Grad H, Wu XY, Friedman S. Antimicrobial substantivity of Chlorhexidine - treated bovine root dentin. *J Endod* 26: 315 - 7, 2000.
20. Lin S, Zuckerman O, Weiss EI, Mazon Y, Fuss Z. Antifungal efficacy of a new Chlorhexidine slow release device to disinfect dentinal tubules. *J Endod* 29: 416-8, 2003.

Author Information

Zahed Mohammadi, DMD, MSD

Assistant Professor, Department of Endodontics, Faculty of Dentistry, Sadoughi University of Medical Sciences