Residual Antibacterial Activity of Minocycline Chlorhexidine and MTAD

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Citation

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Abstract

The purpose of this in vitro study was to compare the antimicrobial substantivity of 2% Chlorhexidine (CHX), MTAD, and 100 mg ml-1minocycline hydrochloride (MH) in human root dentine. One hundred and ten dentine tubes prepared from human maxillary incisors were infected in vitro for 14 days with Enterococcus faecalis. The specimens were divided in to five groups as follows: CHX; MTAD; and MH; infected dentine tubes (positive control); and sterile dentine tubes (negative control). Dentine 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 CHX group and MH 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 CHX was significantly greater than MTAD and MH.

INTRODUCTION

Viable microorganisms remaining after root canal preparation and disinfection contribute significantly to failure in endodontic therapy ([1]). Numerous measures have been described to reduce the numbers of root canal microorganisms, including the use of various instrumentation techniques, irrigation regimens, and intracanal medicaments ([1]). In cases with necrotic pulps as well as in retreatment cases, treatment should be performed in two visits, which is more time-consuming than one-visit treatment ([2]). Furthermore, some studies have suggested that calcium hydroxide is ineffective against E.faecalis ([3]). To overcome the abovementioned problems, an alternative protocol is to use antimicrobial agents that exhibit substantivity, that is, agents that can have a therapeutic effect for a prolonged period.

NaOCl is the most commonly used root canal irrigant, which has both antimicrobial and tissue dissolving properties ([4], [5]).

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 to12 weeks.

Tetracyclines are a group of bacteriostatic antibiotics, which are effective against a wide range of microorganisms ([10]). They readily attach to dentine and are subsequently released without losing their antibacterial activity ([10]). Minocycline (Minocin, Lederle parenterals Inc., USA) was shown to be the most substantive form of the tetracyclines. In endodontics, tetracyclines have been used to remove the smear layer from the instrumented canals, irrigation of the root-end cavities and as an intra-canal medicament.

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. ([10]) showed that MTAD was a more effective disinfectant of the root canal system than 5.25% NaOCl. They also found that the combination of 1.3% NaOCl and MTAD as a final treatment eliminated E. faecalis from human tooth cementum and dentine. ([11], [12]). However, its substantivity has not been yet evaluated. The purpose of this study was to compare the antibacterial substantivity of MTAD, 2% CHX, and minocycline hydrochloride (MH) against E. faecalis in human root dentine in vitro.

MATERIALS AND METHODS

The method used was a modification of the one previously described by Haapasalo and Orstavik ([3]). Fifty intact human

maxillary 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 centre-holed piece of root dentine 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 dentine slices were preserved in vials containing tap water during the procedures to avoid dehydration. The dentine tubes (n=100) 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.

A total of 110 specimens were randomly divided into five groups as follow: Group 1 (30 specimens): 2% CHX, Group 2 (30 specimens): MTAD, Group 3 (30 specimens): MH (Minocin, Lederle parenterals Inc., USA), Group 4 (10 specimens): positive control (infected dentine tubes), Group 5 (10 specimens): negative control (sterile dentine tubes).

Isolated 24-hour colonies of pure cultures of E. faecalis (ATCC 29212) were suspended in 5ml of BHI. The bottles containing each specimen in groups 1,2,3 and 4 were opened under laminar flow. Sterile pipettes were used to remove 2 ml of sterile BHI and to replace it with 2 ml of bacterial inoculum. The bottles were closed and kept at 37oC for 14 days, with the replacement of 1 ml of contaminated BHI for 1 ml of freshly prepared BHI every 2 days, to avoid medium saturation. After the contamination period, each specimen was removed from its bottle under aseptic conditions and the canal was irrigated with 5 ml of sterile saline and dried with sterile paper points. The outer surface of the specimens 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 inserted into the canal lumen with sterile 3-ml plastic syringes and 27-gauge needles until the dentine tubes were totally filled. Ten minutes after placement of irrigants, solutions were removed using sterile paper points. The specimens were then incubated at 37° C for a period of 28 days to maintain humidity. Dentine 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 dentine around the canal. The powder dentine samples obtained with each bur were immediately collected in separate test tubes containing 3 ml of freshly prepared BHI. Thereafter, l00 microlitres from each test tube were cultured on blood agar. 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 bacteria 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 bacteria at all experimental times, which indicated the efficiency of the method. In contrast, negative control group showed no viable bacteria at all experimental times. At all experimental periods, the CHX group and MH group demonstrated the most and least effective antibacterial actions, respectively. The differences between all groups ($p<0.05$) as well as within each group ($p<0.05$) at all periods were statistically significant.

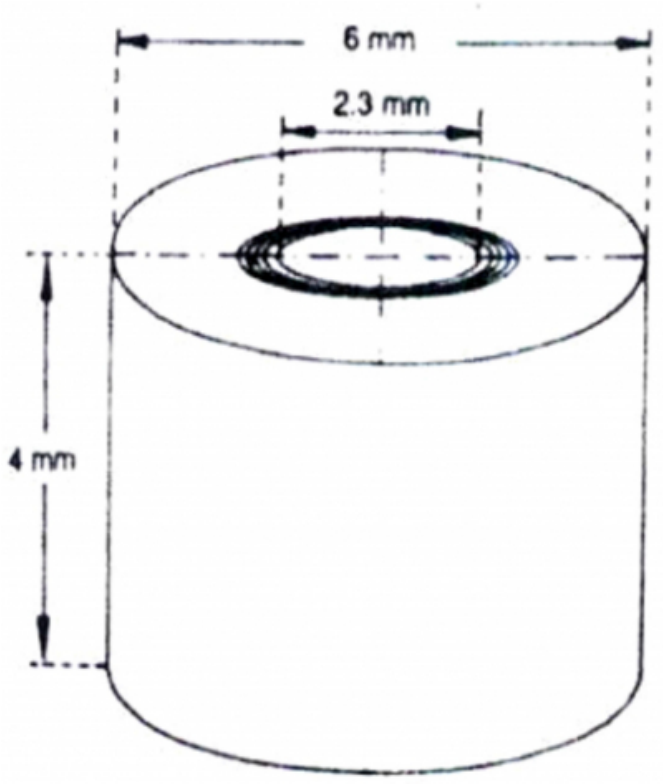
Figure 1

Table 1: Means of the CFU and the Standard Deviation of E. faecalis in experimental groups

	Day 0	Day 7	Day 14	Day 21	Day 28
MH	0.31±0.58	17.16±7.05	34.40±8.79	66.78±10.11	95.25±5.61
MTAD	3.56±3.72	10.35±3.77	14.49±4.67	34.35±4.22	51.53±5.35
CHX	0.27±0.56	4.32±2.21	7.56±2.66	17.21±3.38	38.41±5.43

Figure 2

Figure 1: Schematic view of used dentine tubes (adopted from Gomes et al. (6))



DISCUSSION

Current techniques of debridement leave many areas of the root canal completely untouched by the instruments ([13]). Thus, a root canal irrigant is needed to aid in the debridement of the canals. E. faecalis was chosen for inoculum in this study because it is resistant to intracanal medication with calcium hydroxide ([6]). In the present study, the antibacterial substantivity of CHX, MTAD and MH was monitored for 28 days. The 2% CHX solution had the most effective antibacterial action at all experimental periods.

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. 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 ([14]) 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. ([15]) 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. ([16]) compared the antibacterial substantivity of 2% CHX, 100 mg /ml doxycycline, and 2.6% NaOCl in bovine root dentine 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, dentine should be treated with CHX for 7 days and 5 min. treatment with CHX did not induce substantivity, which is in contrast to our findings. Lin et al. ([18]) attributed the limited antibacterial effect of CHX irrigation to absorb the medication to dentine during the first hour and stated that only after the saturation point after the first hour that the antibacterial capability of CHX increase with time. Stabholz et al. ([19]) found that the antimicrobial substantivity of CHX was significantly lesser than tetracycline HC1 50 mg/ml for 12 days.

CONCLUSION

Under the conditions of the present study, the substantivity of CHX was significantly higher than MTAD and MH.

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