

Original Article

Stability of antibacterial activity of Chlorhexidine and Doxycycline in bovine root dentine

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Received: October 2013

Accepted: December 2013

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ABSTRACT

Objective: The aim of this study was to compare the substantivity of chlorhexidine (CHX) and doxycycline bond to the dentin in diffusion disk method.

Methods: A total of 92 dentin disks were prepared from Bovine's teeth root. After removing cementum and standardizing disks in weight and outer diameter, they were irrigated with sodium hypochlorite 2.5%, ethylenediaminetetraacetic acid 17% and sterile saline and sterilized in autoclave. Then, the first group ($n = 36$) were irrigated with chlorhexidine 2% and the second group ($n = 36$) with doxycycline 100 mg/mL, each for a period of 10 min. The third group ($n = 20$) was considered as the control group. Samples were divided into four subgroups, which after 1 day, 3, 6, and 12 weeks were incubated in plates containing Muller Hinton agar broth and *Enterococcus faecalis* for a period of 48 h at 37°C temperature; then the diameter of the zone of inhibition was measured. The antimicrobial effect of irrigating solutions without binding to the dentin was also studied using the plate well method. One-way and univariate variance tests as well as *Post-hoc* Tukey were used for data analyses.

Findings: The diameter of the zone of inhibition of doxycycline group was significantly more than the CHX group from the beginning to the 3rd week ($P < 0.005$). After 3rd week, mean diameter in doxycycline group declined as compared with the CHX group up to 12th week ($P < 0.005$). The difference between means in two groups was not significant in the 3rd week ($P = 0.87$).

Conclusion: The results of this study show that CHX and doxycycline show antibacterial activity for 12 weeks; although after 3rd week, the substantivity of CHX was significantly greater than doxycycline. Both of these irrigants can be used in other pharmacological and medical fields whenever a long-lasting antibacterial action is needed.

Keywords: Chlorhexidine; Doxycycline; *enterococcus faecalis*; substantivity

INTRODUCTION

Microorganisms play a significant role in development of pulp and periapical diseases, which has been demonstrated in previous studies.^[1,2] Disinfection of the root canal system to reduce the microbial load present in the root canal is one of the main goals of endodontic therapy. Although, mechanical

instrumentation removes a portion of the microbes, but irrigation and intracanal medicaments help more microorganisms and necrotic pulp tissues to be removed.^[3] Calcium hydroxide (Ca[OH]) is commonly used as an intracanal medicament and can predictably disinfect the root canal, but after mechanical removal of Ca (OH), no residual antimicrobial effect could remain.^[4] To obtain more reductions in the microbial load some antimicrobial irrigants such as chlorhexidine (CHX) and Tetracyclines have been used. Unlike sodium hypochlorite (NaOCl),^[5] these irrigants are capable of adsorbing on to the dentin walls, which imparts long-lasting antimicrobial effects, termed substantivity.^[6] CHX is a cationic biguanide that adsorbs on to the cell wall of microorganisms and cause leakage of intracellular components. CHX is effective against both Gram-positive and

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DOI: 10.4103/2279-042X.132705

Gram-negative bacteria.^[7] Some studies have shown 0.02–2% CHX kills the *Enterococcus faecalis* cells in 30 s or less.^[8] *In-vivo* studies found that antimicrobial effect of CHX remains for up to 48 h or even longer period of time.^[9] Tetracyclines are effective against a wide range of microorganisms and other than substantivity show antiresorptive activity and may remove the smear layer from instrumented root canal walls.^[10,11] Due to residual alive micro-organisms in root canal system, which may cause failure of root canal therapy^[12] and since leakage of temporary or final restorations can lead to reinfection of root canal irrigation,^[13-15] using these irrigants seems to be necessary. The aim of this study was to evaluate the antimicrobial efficacy and residual activity (substantivity) of 2% CHX and doxycycline against *E. faecalis* in the bovine root dentin disks in an *ex-vivo* model. Long lasting antibacterial action of such irrigants can prevent the reinfection and may be useful in other pharmacological and medical fields, especially in oral medicine.

METHODS

Ninety-two dentin disks of bovine incisor root were used in this study. First, teeth having complete roots and enough dentine thickness were selected. Crowns were sectioned off from cemento-enamel junction using a diamond saw and tap water irrigation, cementum was removed using polish paper (Matador factory, softlex 240, Germany) and dentin disks were obtained from 1/3 coronal portion of each tooth using a slow speed diamond saw. By caliper (Varnier, caliper 0.02%, China) and analytical balance (R and D Scaltex, [0.001] GR, USA) disks were measured and samples with 7 mm outer diameter and 150-200 mg weight were selected. All samples were preserved in vials containing tap water to avoid dehydration. Disks were individually treated with 5.25% NaOCl (Merck, Germany) and 17% ethylenediaminetetraacetic acid (EDTA) (pH = 7.2) (Meta Diomed, MD-Cleaner, Korea) to remove the smear layer. Samples were divided into three groups: Group A ($n = 36$) were treated by immersion in 2% CHX (Merk, Germany) for 10 min, Group B ($n = 36$) were immersed in doxycycline solution 100 mg/mL and Group C ($n = 20$), as the control group, were treated by immersion in sterile saline for 10 min. All of the disks were then blotted dry paper and after removal of extra liquid they were put in a sterile dish in 37°C. A single colony of *E. faecalis* (ATCC 29272) was selected from blood agar plates and inoculated in brain-heart infusion until it turned turbid. The broth was adjusted to 0.5 McFarland standard equal to 1.5×10^8 bacteria/mm. Muller-Hinton agar plates (Antec diagnosis) were

swabbed with *E. faecalis*. Experimental and control disks were placed on the plates and incubated for 48 h in 37°C. Each of main groups divided to four subgroups. The zones of inhibitions were measured by the average of two perpendicular diameters after 1 day and 3, 6, and 12 weeks.

RESULTS

The zones of inhibitions in two used irrigants were measured and analyzed using two-way analysis of variance. Type of irrigant and the time, both had a significant effect on diameter of zones of inhibitions ($P < 0.001$). Tukey's *Post-hoc* test showed significant differences between CHX and doxycycline. Table 1 demonstrates results of mean diameter of bacterial inhibition zone in two irrigants and control group at four periods of time. The control group revealed inhibition zones and the differences with experimental ones were significant ($P < 0.001$). Except 6th and 12th weeks, there were significant differences between all time periods. The diameter of the zone of inhibition of doxycycline was significantly more than the CHX group from the beginning to the 3rd week ($P < 0.005$). The difference between means in two groups was not significant in the 3rd week ($P = 0.87$) and the mean diameter in doxycycline group declined from the 3rd to 12th week as compared to the CHX group ($P < 0.005$).

DISCUSSION

After cleaning and shaping of the root canal, some of the areas may be untouched and remained bacteria may cause failure of treatment; thus a suitable irrigant is needed to aid in debridement of the canals and killing of bacteria. In this study, *E. faecalis* was chosen

Table 1: Means of zone of inhibition of *E. faecalis* in experimental and control groups

Irrigant	Time since study initiation	N	Zone of inhibition (mm)
Chlorhexidine	1 day	9	13.84±0.65
	3 weeks	9	14.47±0.38
	6 weeks	9	14.87±0.53
	12 weeks	9	14.94±0.45
Doxycycline	1 day	9	17.56±0.35
	3 weeks	9	13.71±0.35
	6 weeks	9	11.23±0.38
	12 weeks	9	11.60±0.66
Sterile saline (control group)	1 day	5	8.80±0.27
	3 weeks	5	8.72±0.48
	6 weeks	5	8.60±0.4
	12 weeks	5	7.0±0

Data presented as Mean ± SD. *E. faecalis*=*Enterococcus faecalis*, SD=Standard deviation

as the test organism because it is well-recognized as a pathogen associated with persistent apical periodontitis in endodontically treated teeth and is resistant to intracanal medication with Ca (OH).^[16] Antibacterial substantivity of CHX and doxycycline is compared for 12 weeks in this study. Although doxycycline has shown more effective antibacterial action than CHX in 1st day, its effect suddenly drops off after 3 weeks and show constant level, which is lower than CHX at 6th up to 12th weeks. CHX shows less antibacterial effect at 1st day than doxycycline, but its effect increases in next periods. Hence, it seems that CHX has more substantiative antimicrobial activity that lasts up to 12th week. High level of antibacterial activity of doxycycline disks at the beginning which continues up to 3rd week shows that doxycycline substantivity decreases after 3 weeks and hence that less antibacterial activity of the disk is shown up to 12th week. In contrast, CHX disks show low level antibacterial activity at 1st day, which increases up to 12th week. Zone of inhibition in control groups (disks without use of irrigants) may be a result of residual hypochlorite and EDTA used in previous steps. The finding of the present study showed 2% CHX and doxycycline both have substantiative antimicrobial activity that lasts up for 12 weeks. These results are similar to the results of Mohamadi^[17] and Rosenthal^[18] study which showed CHX substantivity for 12 weeks, and complete the result of White study^[6] or *in-vivo* study of Leonardo and Shahani, which had shown antibacterial activity of CHX just up to 72 h and 48 h.^[19,20] Although, Lenet and komorowski have reported that for induction of substantivity, dentin should be treated for 7 days,^[21,22] but in the present study, 10 min contact of dentin with doxycycline or CHX has been shown to be enough to make substantivity. Our results showed that doxycycline up to 3rd week is more potent antibacterial irrigant than CHX; however, Stabholz found that antimicrobial substantivity of tetracycline was significantly greater than CHX for 14 days and CHX didn't show antibacterial effect even as subgingival irrigant.^[23,24] After 6 weeks, CHX antibacterial effect increases and becomes more than doxycycline. Rasmik showed that half-life of CHX is more than doxycycline (14 weeks against 3 weeks).^[25] In other studies, substantivity of CHX has been continued up to 28 days and in all periods, CHX has been more potent than doxycycline.^[26] The point to make is the effect of different methods used in these two studies. For instance, in the latter study canals were initially infected by bacteria and then treated with irrigants and antibacterial activity was measured by culturing and colony forming unit counting, but when dentin is exposed to irrigants before bacterial infection, as used in the recent study, it removes errors of sampling

and culturing. In addition, this method resembles secondary infections after leakage of final or temporary restorations. Because antibacterial effect of CHX and doxycycline is affected by factors like local pH and endodontic sealers can change the pH, so clinical relevance of the results is limited, autoclaving might denature dentin collagens and reduce the retention of CXH and doxycycline.

Finally, results of this study show that CHX and doxycycline are suitable irrigants against *E. faecalis* with substantivity, but CHX show more antibacterial effect than doxycycline after 3 weeks and it means that CHX is more substantiative antimicrobial factor.

AUTHORS' CONTRIBUTION

All authors contributed the idea of research, design of study, data analysis and manuscript preparation.

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How to cite this article: Khademi AA, Saleh M, Khabiri M, Jahadi S. Stability of antibacterial activity of Chlorhexidine and Doxycycline in bovine root dentine. *J Res Pharm Pract* 2014;3:19-22.
Source of Support: Nil, **Conflict of Interest:** None declared.