Original Article

Antibacterial Substantivity of Three Concentrations of Doxycycline on Bovine Root Dentin Infections: an In Vitro Study

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ABSTRACT

Introduction: The aim of this in vitro study was to compare the antimicrobial activity and substantivity of three concentrations of doxycycline on bovine root dentin infections.

Methods and Materials: Seventy dentin tubes prepared from intact bovine incisors were infected in vitro for 14 days with Enterococcus faecalis. The specimens were divided into five groups according to the used intracanal irrigation as follows: Group1: l00mg/ml of doxycycline hydrochloride solution (DHS) (n=20); Group 2: 50mg/ml of DHS (n=20); Group 3: 10mg/ml of DHS (n=20); Group 4: positive control (infected dentin tubes) (n=10); and Group 5: sterile saline (negative control) (n=10). 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. After culturing, the numbers of colony-forming units (CFUs) were counted. Data were analyzed 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 numbers of CFUs in all three experimental groups were minimum in first cultures, and the obtained results were significantly different from each other at any time period ($P<0.05$). In first culture, the groups 1 (0.400 ± 0.699) and 3 (4.700 ± 3.683) showed the lowest and highest numbers of CFUs, respectively. In each group, the numbers of CFUs increased significantly by time lapse ($P<0.05$).

Discussion: Under the conditions of this study, it can be concluded that doxycycline HCl may be useful as a substantive antimicrobial agent.

Key Words: Doxycycline HCL, Enterococcus faecalis, Substantivity.

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Introduction

Viable microorganisms remaining after root canal preparation and disinfection significantly contribute to failure in endodontic therapy [1]. Several methods including mechanical instrumentation, using irrigation solutions, and intracanal medicaments have been described to reduce root canal microorganisms [1]. In cases with necrotic pulps as well as in retreatment cases, treatment should be done in 2 visits, which is more

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time-consuming than one-visit treatment [2]. Further, some studies have suggested that calcium hydroxide is ineffective on Enterococcus faecalis [3]. To overcome the mentioned problems, an alternative protocol is necessary to use substantive antimicrobial agents [4-6].

Tetracyclines are effective against a wide spectrum of microorganisms [7-9]. Tetracyclines readily attach to dentin and are subsequently released without losing their antibacterial activity [10]. In endodontics, tetracyclines have been used to remove the smear layer from the instrumented canals (8,9), irrigation of root – end cavities [11], and as an intracanal medicament [12]. The aim of this study was to compare the antimicrobial substantivity of three concentrations of doxycycline in bovine root dentin infections.

Methods and Materials

The used method was a modification of the one which was previously described by Haapasalo and Orstavik [13]. Thirty intact bovine central incisors were selected for this study. The specimens were kept in 0.5% sodium hypochlorite solution for no longer than seven days. The apical 5 mm and two-thirds of the crown were removed from each tooth with a water-cooled rotary diamond saw at 1000 rpm (Isomet Plus precision saw, Buehler, IL, USA). Cementum was removed using polish paper (Ecomet 3, variable-speed grinder-polisher, Buehler, IL, USA), which resulted in a centre-holed piece of root dentin with outer diameter of 6 mm. 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 (figure 1). All teeth and dentin slices were preserved in vials containing tap water during the procedures to avoid dehydration. The dentin tubes (n=80) were individually treated with 5.25% NaOCl, and 17% EDTA (pH=7.2), each for five minutes to remove the smear layer [13]. The specimens were then placed in Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, UK) and autoclaved. They were then kept in an incubator at 37oC for 24 hours to check the efficacy of the sterilization. A total of 80 specimens were randomly divided into five groups as follow: Group 1 (20 specimens): 100-mg/ml of doxycycline HCl; Group 2 (20 specimens): 50-mg/ml of doxycycline HCl; Group 3 (20 specimens): 10-mg/ml of doxycycline HCl; Group 4 (10 specimens): positive control (infected dentin tubes); and Group 5 (10 specimens): negative control (sterile dentin tubes).

Isolated 24-hours colonies of pure cultures of E. faecalis (ATCC 29212) were suspended in 5ml of BHI broth. The bottles containing each specimen in groups 1,2,3,4 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. 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 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-wells 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 irrigation solutions, they were removed using sterile paper points. Then, specimens were incubated at 37°C for a period of 28 days to maintain their 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, 4, 21, and 28 days. Each bur removed approximately 0.1 mm of dentin around the canal. The powdered dentin samples ob-

tained with each bur were immediately collected in separate test tubes containing 3 ml of freshly prepared BHI. Thereafter, l00 micro liters from each test tube were cultured on blood agar. Growing colonies were counted and recorded as CFU. Results were analyzed 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 the 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 CFUs obtained from five consecutive dentinal layers is presented in table1. The number of CFUs in all three experimental groups was minimal in the first cultures. The positive control group showed viable bacteria at all experiment times, which indicate the efficiency of the method. On the other hand, negative control group showed no viable bacteria at all experiment times. At all experimental periods of time, the 100mg/ml group demonstrated the most effective antibacterial action ($P<0.05$).

Table 1. Enterococcus faecalis CFUs in experimental groups (mean±SD).

	Day 0	Day 7	Day 14	Day 21	Day 28
100mg/ml	0.40±0.69	4.66±2.34	9.70±2.75	20.20±3.22	44.44±5.52
50mg/ml	0.50±3.97	9.00±3.74	15.40±4.55	37.00±5.33	59.66±5.36
10mg/ml	4.70±3.68	16.11±8.05	37.40±8.99	61.80±11.11	88.55±5.50

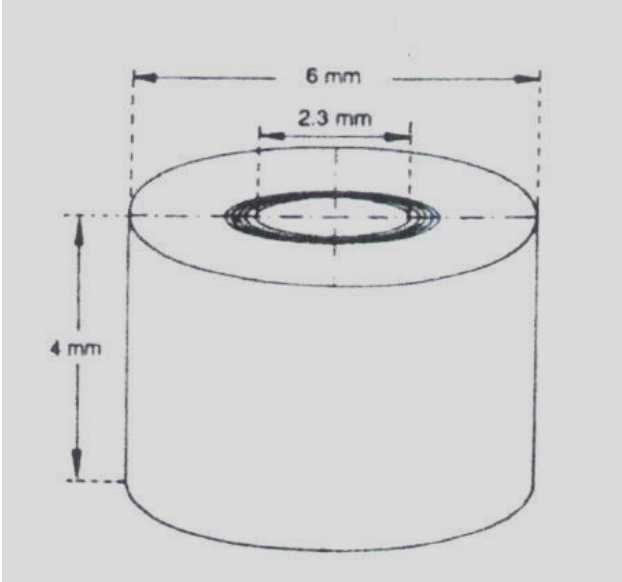

Figure1. Schematic view of used dentin tubes (adopted from Gomes et al (14))

Discussion

Microorganisms remaining in the irregular spaces of the root canal system (RCS) are the main etiological factor for the failure of root canal therapy (12). The main objectives of root canal therapy are the removal of diseased tissue, elimination of microorganisms present in the canals and dentinal tubules, and prevention of recontamination after treatment. Current techniques of root canal debridement leave many areas of the RCS completely untouched by the instruments. Thus, root canal irrigation is needed to aid in debridement of the canals [4].

In this study, bovine incisor teeth were used because of their dentinal tubules which are very similar to human teeth in quantity, size, diameter, morphology, and density [14]. Also, E. faecalis was chosen for inoculation, because it is considered resistant to the common intracanal medication with calcium hydroxide and is frequently associated with persistent disease after endodontic treatment. Because effective alternative therapeutic modalities against E. faecalis are desirable, the use of this particular microbe is appropriate to test potential therapeutic modalities. In addition, E. faecalis is a non-fastidious microbe that is relatively easy to culture and that has been implemented successfully in most studies by using the original model [14].

Tetracyclines are broad-spectrum bacteriostatic antibiotics [7]. Doxycycline is a hydroxy-derivative of tetracycline. The acidic property (pH=2) of tetracycline HCl is probably responsible for the breakdown of smear layer [9]. Tetracyclines, including doxycycline, readily attach to dentin and are subsequently released without losing their antibacterial activity. This property creates a reservoir of active antibacterial agent, which is released from dentin surfaces in a slow and sustained manner [9].

In the present study, doxycycline HCl, 100 mg/ml, showed the highest antibacterial substantivity at all time periods. Stabholz et al assessed the antimicrobial substantivity of human root surfaces after in-situ subgingival irrigation with tetracycline HCl or chlorhexidine. Their results demonstrated that the antimicrobial substantivity of tetracycline HCl, 50 mg/ml, was significantly greater than chlorhexidine digluconate for 12 days and greater than saline for 16 days [10]. Demirel et al evaluated the antibacterial substantivity of topically applied doxycycline hydrochloride on root surfaces obtained from patients with periodontal disease. They found that antibacterial efficiency of specimens treated with the concentration of 100mg/ml persisted for 14 days, but antibacterial efficiency of specimens treated with the concentration of 50mg/ml persisted for 7 days. So, they suggest that concentration of 100mg/ml might be the most reasonable one to be used for topical application in clinical trials [15]. Barkhordar et al assessed the substantivity of three concentrations of doxycycline hydrochloride (100mg/ml, 50mg/ml, and 25mg/ml) on smear layer on intracanal walls. They found that doxycycline hydrochloride of 100mg/ml was the most effective in removing the smear layer [8]. Considering the results of the present and above mentioned studies, it should be noted that while doxycycline possesses antimicrobial substantivity, its use should be considered as a final rinse, prior to root canal obturation, because of its lack of tissue dissolving ability.

Also, it can be concluded that under the conditions of the present study, the antimicrobial substantivity of 100 mg/ml of doxycycline was significantly greater than its lower concentrations.

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