

The Effect of Ascorbic Acid on the Substantivity of Tetraclean in Sodium Hypochlorite-Treated Bovine Dentin

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Abstract

Objective: The purpose of this study was to assess the *in vitro* effect of ascorbic acid on the antibacterial substantivity of Tetraclean in bovine root dentin pretreated with sodium hypochlorite (NaOCl).

Materials and Methods: Eighty dentin tubes prepared from bovine incisor teeth were infected with *Enterococcus faecalis* for 14 days. The specimens were divided into five groups as follows: Tetraclean; 5.25% NaOCl/Tetraclean; 5.25% NaOCl/ascorbic acid/Tetraclean; infected dentin tubes (positive control); and sterile dentin tubes (negative control). At experimental times of 0, 7, 14, 21 and 28 days, dentin chips were removed from the canals by sequential sterile low-speed round burs with increasing diameters of 025, 027, 029, 031 and 033 ISO sizes, respectively. After culturing, the number of colony-forming units (CFU) was counted.

Results: In all experimental groups, the number of CFU was minimum in the first cultures and the results obtained were significantly different at any time period ($p < 0.05$). The Tetraclean group showed the most effective antibacterial action at all five experimental periods ($p < 0.05$). NaOCl/Tetraclean group showed the least antibacterial activity at all time periods. The NaOCl/ascorbic acid/Tetraclean group showed similar antibacterial substantivity to the Tetraclean group at all time periods ($p > 0.05$).

Conclusion: Ascorbic acid prevents the decrease of residual antibacterial activity of Tetraclean in dentin samples pretreated with NaOCl.

Keywords: Ascorbic Acid; Enterococcus Faecalis; Pretreatment; Sodium Hypochlorite; Substantivity; Tetraclean

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INTRODUCTION

The success of root canal treatment depends on the eradication of microorganisms from the

root canal system and prevention of re-infection [1]. Due to its complex anatomy, with multiple fins, isthmuses, ramifications and

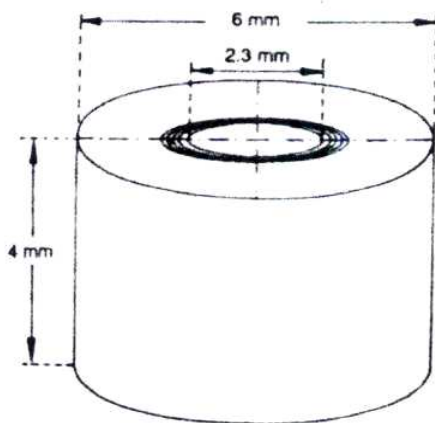


Fig1. Schematic view of used dentin tubes (adopted from Mohammadi and Shahriari (10))

accessory canals, it is virtually impossible for mechanical instrumentation to shape and clean the entire root canal system [2]. According to a study conducted by Byström and Sundqvist, the number of intracanal microorganisms reduced 100-1000 fold following hand instrumentation with stainless steel files and using sterile saline as a root canal irrigant. However, bacteria were not eliminated completely. [3]. Thus, using an appropriate irrigation solution is essential to remove residual tissue and to kill microorganisms.

Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigation solution. The antimicrobial effectiveness of NaOCl is based on its high pH (hydroxyl ions action). The high pH of NaOCl disturbs the completeness of the cytoplasmic membrane via inhibiting enzymes irreversibly, biosynthetic changes in cellular metabolism and depriving phospholipids observed in peroxidation of lipids [4]. Furthermore, NaOCl possesses excellent tissue dissolving ability [4].

Tetracyclines have a wide-range antimicrobial activity [5]. Furthermore, they have residual antimicrobial activity. In other words, tetracyclines are absorbed to dentin immediately and are delivered thereafter without missing their antibacterial effectiveness [5]. In the field of

endodontics, tetracyclines have been used to irrigate root-end cavities [6], to remove the smear layer from the instrumented canals [7] and as an intra-canal medicament [8]. Currently, there are two antibiotic-based root canal irrigation solutions in the field of endodontics [5]. MTAD is composed of doxycycline, citric acid and Tween 80. [9]. MTAD has been shown to have a clinically effective antibacterial activity [5]. In addition, its antibacterial substantivity has been proved in some studies [10, 11]. The other antibiotic-based irrigant, Tetraclean, (Ogna Laboratori Farmaceutici, Muggiò, Italy), similar to MTAD, is a mixture of an antibiotic, an acid and two detergents (propylene glycol and cetrimide) [12]. However, the concentration of the antibiotic, doxycycline (50 mg mL^{-1}) and the type of detergent (polypropylene glycol and cetrimide) differ from those of MTAD [12]. Recently, Mohammadi *et al.* showed that the substantivity of Tetraclean was significantly greater than MTAD and 5.25% NaOCl [13].

MTAD as well as Tetraclean do not dissolve organic tissues [14]. Therefore, NaOCl should be used to dissolve organic tissues. Tay *et al.* [15] showed that the antimicrobial substantivity of MTAD was reduced when MTAD was applied to 1.3% NaOCl-irrigated dentin. Recently, Mohammadi *et al.* [16] showed that substantivity of Tetraclean was reduced in NaOCl-irrigated dentin. A study demonstrated that pretreatment of NaOCl-irrigated dentin with ascorbic acid prevented the decrease in MTAD substantivity [17]. Considering the closed similarity between the composition of MTAD and Tetraclean, the aim of this study was to assess the effect of pretreatment of NaOCl-irrigated dentin with ascorbic acid on the substantivity of Tetraclean.

MATERIALS AND METHODS

The method of the present study was modified from that described by Haapasalo and Ørstavik [18]. Intact bovine central incisor teeth were used. In order to clean and disinfect the sur-

face, the teeth were kept in 0.5% NaOCl solution (Merck, Germany) for up to 7 days. The clinical crown and apical third were removed from each tooth using a rotary diamond saw at 1000 rpm (Isomet Plus precision saw, Buehler, IL, USA) under water-cooling.

The cementum was removed using polish paper (Ecomet 3, variable-speed grinder-polisher, Buehler, IL, USA), which resulted in a center-holed piece of root dentin with a 6-mm outer diameter (Fig 1).

Using a diamond saw, the remained piece of each tooth was cut into 4-mm-thick slices.

Using an ISO 023 slow speed round bur, the canals of the 4-mm blocks were enlarged (standardized).

During the procedures, all teeth and dentin slices were preserved in vials containing tap water to prevent dehydration. In order to remove the smear layer, each dentin block was placed inside 3 ml of 17% EDTA with a pH of 7.2 placed in an ultrasonic bath for 10 min and irrigated under tap water for 1 hour.

Thereafter, the mentioned process was conducted with 5.25% NaOCl (Merck, Darmstadt, Germany) instead of EDTA.

The specimens were then placed in BHI broth (Oxoid, Basingstoke, UK) and autoclaved at 121 C with 1 atm pressure for 15 minutes.

The specimens were kept in an incubator at 37°C for 24 h to monitor the efficacy of the sterilization. A total of 80 specimens were randomly divided into five groups as follows:

Group 1 (20 specimens): Tetraclean (Ogna Laboratori Farmaceutici, Muggiò, Italy) irrigation

Group 2 (20 specimens): 5.25% NaOCl and Tetraclean irrigation

Group 3 (20 specimens): 5.25% NaOCl, ascorbic acid and Tetraclean irrigation

Furthermore, 10 sterile and 10 infected dentin tubes were used as negative and positive control groups, respectively. In order to contaminate the dentin tubes, isolated 24-h colonies of pure cultures of *E. faecalis* (ATCC 29212) suspended in 5 ml of BHI were used. The bottles containing each specimen in groups 1, 2, 3 and 4 were opened under laminar flow. Two milliliters of sterile BHI was removed with sterile pipettes and replaced with 2 ml of bacterial inoculum. The bottles were closed and kept at 37°C for 14 days. To prevent medium saturation, 1 ml of freshly prepared BHI was replaced by 1 ml of contaminated BHI every 2 days. Each specimen was removed from its bottle under aseptic conditions after 14 days and the canal was irrigated with 5 ml of sterile saline and dried with sterile paper points.

Table 1. Mean of the CFU and the standard deviations of *E. faecalis* in the experimental groups

	Day 0	Day 7	Day 14	Day 21	Day 28
Tet	0.00±0.00	0.00±0.00	0.37±0.65	6.68±2.59	15.35±3.21
SH/Tet	0.20±0.49	4.52±2.24	7.74±2.84	17.28±3.64	34.49±4.54
SH/AA/Tet	0.00±0.00	0.10± 0.00	0.40±0.72	6.79±3.14	16.24±5.64

Tet =Tetraclean; SH=sodium hypochlorite; AA=ascorbic acid

The outer surface of the specimens was covered with two layers of nail varnish to prevent contact of the medicament with the external surface. Afterwards, specimens were fixed at the bottom of wells of 24-well cell culture plates using decontaminated sticky wax, which also obliterated the apical surface of the root canal. Finally, the irrigating solutions were inserted into the canal lumen with sterile 3-ml plastic syringes and 27-gauge needles until the dentin tubes were totally filled.

Solutions were removed using sterile paper points ten minutes after placement into the lumen. In group 2 samples, dentin tubes were firstly filled with 5.25% NaOCl for 10 minutes. The NaOCl solution was then removed with sterile paper points and dentin tubes were filled with Tetraclean for 10 minutes.

However, in group 3 samples, dentin tubes were filled with 10% ascorbic acid solution for 10 minutes before using Tetraclean and after removing 5.25% NaOCl solution.

The specimens were then incubated at 37°C for 28 days to maintain humidity. At experimental times of 0, 7, 14, 21 and 28 days, dentin chips were removed from the canals with sequential sterile low-speed round burs with increasing diameters of 025, 027, 029, 031 and 033 ISO sizes, respectively. 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 BHI. Thereafter, 100 µL from each test tube was cultured on blood agar. Growing colonies were counted and recorded as CFU.

RESULT

The results were presented as mean Log₁₀ CFU. The positive control group showed viable bacteria at all experimental times, which indicated the efficiency of the method. The average number of CFU in each specimen of this group was 120. In contrast, the negative control group showed no viable bacteria at all experimental times. The number of CFU was

minimum in the first cultures (day 0) in all experimental groups and there were statistically significant differences between the obtained results at any time period ($p = 0.03$). The Tetraclean group showed the most effective antibacterial action at all five experimental periods ($p < 0.001$). The NaOCl/Tetraclean group showed the least antibacterial activity at all time periods. The NaOCl/ascorbic acid/Tetraclean group showed similar antibacterial substantivity to the Tetraclean group at all time periods ($p = 0.002$).

DISCUSSION

Enterococcus faecalis was chosen for the following reasons: (i) its prevalence in root-filled teeth with periradicular lesions using culturing and polymerase chain reaction (PCR) methods is 24-70% and 67-77%, respectively [19]. (ii) Its capacity to endure prolonged periods of starvation until an adequate nutritional supply becomes available has been demonstrated [19]. (iii) It can induce infection separately (as mono-infection) [19]. The method of the present study was a modification of that proposed by Haapasalo and Orstavik [18]. Using the mentioned method, each consecutive bur size removes only 0.1 mm of the dentin thickness around the root canal. According to the microcomputed tomography (CT) studies, comparatively huge areas of the root canal surfaces leave untouched by files [20]. Therefore, using an irrigation solution is necessary to improve the debridement of the canals. For improvement of the efficacy of root canal irrigants, the irrigants must be in contact with the dentin walls and debris [21]. The closeness of this approximation is related to the wettability of the irrigant on dentin surface and wettability of irrigants has a strict correlation with their surface tension [21]. The surface tension is defined as “the force between molecules that produces a tendency for the surface area of a liquid to decrease” [22].

Surface tension is the force between molecules that decreases the surface area of the irrigant.

Endodontic irrigants should have very low surface tension. This force reduces the penetration depth of the liquid into a capillary tube [23]. Enhancing the wettability of an antimicrobial irrigant increase its protein solubility and improves its efficacy in the uninstrumented parts of the root canal [23]. Despite the strong antimicrobial activity of NaOCl against *E. faecalis*, even in the form of biofilm [4], its high surface tension [24] does not allow it to reach bacterial stocks in the depth of dentinal tubules and canal irregularities. To overcome this problem, some antibiotic-based irrigation solutions (i.e. MTAD and Tetraclean) with low surface tension have been introduced. New generation of endodontic irrigants combine a powerful detergent effect with a strong antimicrobial efficacy.

Therefore, it is recommended to use NaOCl with MTAD or Tetraclean. In fact, in the present study, the effect of ascorbic acid (vitamin C) was assessed on the reversing the oxidative potential of NaOCl as a reducing agent. Findings demonstrated that treatment of NaOCl-irrigated dentin with 10% ascorbic acid for 10 minutes prevents reduction of the substantivity of Tetraclean by prohibiting oxidation of doxycycline by NaOCl. Reducing the activity of ascorbic acid has been demonstrated previously [25, 26].

Ascorbic acid also prevents photodegradation of tetracycline isomers (doxycycline and minocycline) [27, 28]. Using a human dentin tube model, Mohammadi and Shahriari [10] demonstrated that residual antibacterial activity of MTAD was significantly greater than CHX. In another study using bovine dentin tube model, Mohammadi et al. [13] demonstrated that the residual antibacterial activity of Tetraclean was significantly greater than MTAD and 5.25% NaOCl. Another study demonstrated that the residual antibacterial activity of Tetraclean was significantly greater than Hypoclean (a NaOCl-based irrigation solution with reduced surface tension) and

5.25% NaOCl [29]. Torabinejad et al. [30] demonstrated that when MTAD was used as initial rinse followed by the use of various concentrations of NaOCl as final rinse, a chemical reaction happened between NaOCl and the residual MTAD, which caused formation of a brown solution in the root canal. They proposed that such a phenomenon might be caused by the dentinal absorption and release of doxycycline present in MTAD and the final rinse of NaOCl [30].

The reaction of NaOCl with either doxycycline or citric acid in MTAD is not an acid-base reaction. Rather, it is a redox reaction that involves the oxidation of doxycycline by NaOCl [17]. Tay et al. [15] examined the effect of NaOCl-MTAD interaction on the antimicrobial substantivity of MTAD in dentin. Findings demonstrated that the use of 1.3% NaOCl as an initial rinse resulted in a 30% reduction in the substantivity of MTAD. This study [15] also showed that treating the NaOCl-irrigated dentin with ascorbic acid prevented the interaction between NaOCl and MTAD. In another study, Mohammadi et al. [16] found that substantivity of Tetraclean in NaOCl-treated dentin reduced significantly. The reduction of the substantivity of Tetraclean and MTAD can be attributed to the partial loss in the substantivity from the doxycycline component.

CONCLUSIONS

Within the limitations of the present study, in NaOCl-irrigated dentin, ascorbic acid prevented the decrease in the substantivity of Tetraclean.

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