

Efficacy of Calcium Hydroxide: Chlorhexidine Paste as an Intracanal Medication in Bovine Dentin

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The purpose of this study was to evaluate the antibacterial efficacy of an intracanal medication composed of calcium hydroxide with 2% chlorhexidine. Dentin from 24 bovine incisors was used. The incisors were made into standardized cylindrical segments of dentin and infected with *Enterococcus faecalis*. They were then treated with an intracanal paste composed of calcium hydroxide and sterile water or an intracanal paste composed of calcium hydroxide and 2% chlorhexidine for 1 week. Dentin shavings were collected, suspended in solution, and spread on brain-heart infusion agar. After incubation, colony-forming units were enumerated. The amount of bacteria per mg of dentin was determined. The calcium hydroxide paste with 2% chlorhexidine was significantly more effective at killing *E. faecalis* in the dentinal tubules than calcium hydroxide with water.

One of the goals of endodontic therapy is the reduction or elimination of bacteria and their by-products from the root canal system. Proper cleaning, shaping, and irrigation have been shown to significantly reduce and sometimes eliminate bacteria from canals (1). The use of intracanal medications to disinfect the root canal system has been advocated (2). Reasons for the use of intracanal medications are: (a) to eliminate bacteria in the root canal; (b) to prevent bacterial proliferation between appointments; and (c) to act as a physiochemical barrier, preventing root canal reinfection and nutrient supply to the remaining bacteria (3). Calcium hydroxide has been the intracanal medication of choice (4). Calcium hydroxide has been demonstrated to improve dissolution of the pulp tissue by sodium hypochlorite (NaOCl) and provide antimicrobial activity (2, 5). However, the antimicrobial activity of calcium hydroxide seems dependent upon direct contact with bacteria (4). Haapasalo and Ørstavik (6) have demonstrated that it is not effective in eliminating bacteria from dentinal tubules.

Recently, chlorhexidine has been shown to be an effective antimicrobial endodontic irrigant (7, 8). However, chlorhexidine does not have the ability to dissolve tissue, and gels containing chlorhexidine can be difficult to remove from the canal space.

White et al. (9) and Leonardo et al. (10) demonstrated the property of substantivity for chlorhexidine. They demonstrated that chlorhexidine irrigation continued to prevent reinfection of dentin for up to 72 h. Several investigators have demonstrated the antimicrobial effectiveness of chlorhexidine gel or sustained release vinyl ribbons containing chlorhexidine as intracanal medications (11, 12). The purpose of this investigation was to evaluate a paste made of calcium hydroxide mixed with 2% chlorhexidine for antimicrobial efficacy against *Enterococcus faecalis* in the dentinal tubules of bovine incisors.

MATERIALS AND METHODS

Twenty-four bovine incisors were extracted and prepared according to the methods of Haapasalo and Ørstavik (6) as modified by Lenet et al. (12). The incisors were placed in 5.25% NaOCl after extraction to remove any soft tissue. The cementum was removed from the root surface using a Dremel tool (Dremel, Racine, WI) with a cylindrical stone rotating at 3000 rpm. This produced a cylinder of bovine dentin with an external diameter of 7 mm. The apical portion of the root was then removed using a disc in a slow-speed handpiece. The internal diameter of the root canal space was prepared to a size #10 round bur (Brasseler USA, Savannah, GA, U.S.A.) using a dental lathe. Then the crowns of the incisors were removed and the roots were cut into 4-mm long segments using a disc in a slow-speed handpiece. To remove the smear layer, the segments of dentin were placed in an ultrasonic bath of 17% EDTA followed by 5.25% NaOCl for 5 min each. The segments of dentin were sterilized by autoclaving at 121°C for 30 min. Under aseptic conditions, the negative controls were coated with sticky wax. The segments were placed in tryptic soy broth (Becton Dickinson, Cockeysville, MD, U.S.A.) containing a culture of *E. faecalis* and incubated for 5 days at 35°C to infect the dentinal tubules (13). After 5 days, the segments were removed from the broth. Each segment was rinsed with 2 ml of sterile water, blotted dry with sterile gauze, and divided into 2 groups of 10. The segments were glued upright to Petri dishes using a quick-setting epoxy. The canal space of each segment was then filled with a paste of calcium hydroxide and sterile water (group A) or a paste of calcium hydroxide and 2% chlorhexidine (group B). The segments were again incubated at 35°C and 100% humidity for 1 week. After 1 week, the segments were removed from the Petri dishes and the paste was removed using 2 ml of sterile water

irrigation. To test for bacterial survival, dentin shavings from within the canal were collected using round burs of increasing diameter within the canal. Burs, size 29, 31, 33, and 35 (Brasseler USA), were used to create dentin shavings from the inner portion of the segments; burs 37, 40, 42, and 45 were used to create dentin shavings in the outer portion of the segments. The dentin shavings were collected on sterile aluminum foil and weighed. The dentin shavings were suspended in a solution of reduced transport fluid (14). A 1:50 dilution of the solution was made and 100 μ l of the solutions were spread on brain-heart infusion agar plates (Becton Dickinson). They were incubated for 24 h and colony-forming units (CFU) were enumerated. Using the recorded weight of dentin shavings, the number of CFU/mg of dentin was determined.

Independent *t* tests were used to compare the total number of CFU/mg of dentin in both groups. A paired *t* test was used to compare the number of colonies from the inner and outer layers of dentin within each segment.

RESULTS

The total number of CFU of *E. faecalis* per mg of dentin shavings from group A (mean 1040 \pm 3134 CFU/mg) was significantly ($p < 0.001$) different from group B (mean 23 \pm 41 CFU/mg). The calcium hydroxide paste with 2% chlorhexidine was more effective at killing *E. faecalis* in the dentinal tubules than calcium hydroxide with water.

The difference in the number of CFU between the inner and outer layers of each segment was not significant. There was no growth from the negative control dentin segments.

DISCUSSION

Bovine dentin is readily available, and the segments can be easily standardized. In addition, bovine dentin has been demonstrated to be similar to human dentin in structure, composition, and number of tubules (6, 13). It is believed that there was no significant difference in the number of CFU between the inner and outer layers of dentin in this study because the cementum was removed from the outer surface, allowing *E. faecalis* to penetrate and grow throughout the length of the tubules. *E. faecalis* was chosen as the test organism because it was previously shown to infect tubules rapidly and persist within the tubules for at least 10 days without nutrient supply (6, 13). *E. faecalis* has also been demonstrated to be involved in failing root canal therapy (15). *E. faecalis* has been shown to be resistant to killing by calcium hydroxide medications (2, 16, 17). A 2% solution of chlorhexidine was used in this study because chlorhexidine has been demonstrated to be antimicrobial when used as an irrigant or an intracanal medication (7–10, 12, 13, 18). It seemed likely that an additive or synergistic antimicrobial effect might result from the mixture of calcium hydroxide with chlorhexidine. CFU were used because they allow quantification of bacteria per milligram of dentin (19). Without enumeration of CFU, it would only be possible to state whether there were culturable bacteria present.

Peters et al. (19) stated, "There is no evidence that special measures should be taken to kill the bacteria in the dentinal

tubules." It can be argued that bacteria located within tubules after obturation become "entombed" and are of little consequence unless they have access to nutrients. However, in cases that are resistant to therapy, bacteria and their by-products are likely to be the cause for lack of healing. Trope et al. (20) found that healing rates rose when intracanal calcium hydroxide was used. This study demonstrated that when calcium hydroxide is mixed with chlorhexidine, the antimicrobial efficacy of the mixture is greater than calcium hydroxide by itself.

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