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Influence of Different Vehicles on the Antibacterial Effects of Calcium Hydroxide

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This study evaluated the influence of three different vehicles on the antibacterial activity of calcium hydroxide against four bacterial species commonly found in endodontic infections. For this purpose, a broth dilution test using 24-well cell culture plates was performed. Results showed that all pastes were effective in killing the bacteria tested, but at different times. The calcium hydroxide/camphorated paramonochlorophenol/glycerin paste was the most effective against the four bacterial strains tested.

In long-standing endodontic infectious processes, bacteria may propagate to the entire root canal system, including ramifications, isthmi, fins, and dentinal tubules. In these regions, bacteria are usually unaffected by the instruments and irrigants used in the chemomechanical preparation. Studies have reported that bacteria may remain viable even after complete chemomechanical preparation and thereby jeopardize the outcome of the root canal treatment (1, 2).

The use of intracanal medications has been advocated in the treatment of pulpless teeth for the following reasons: (a) eliminate surviving bacteria in the root canal; (b) prevent bacterial proliferation between appointments; and (c) act as a physicochemical barrier, preventing root canal reinfection and nutrient supply to the remaining bacteria (3). Because medications remain for a longer period of time in the canal than irrigants, they may reach bacteria located in inaccessible regions of the root canal system.

Calcium hydroxide has been widely used in endodontics as an intracanal medication. This strongly alkaline substance has a high antibacterial activity against oral bacteria. Studies have demonstrated that calcium hydroxide is superior to camphorated paramonochlorophenol (CPMC) in effectiveness on anaerobic bacteria (4, 5). However, its effects are highly dependent on the availability of hydroxyl ions in solution. Culture media, tissue fluids, and dentin possess buffer substances that can prevent calcium hydroxide activity by causing a pH drop (6). This statement is reinforced by findings from studies evaluating the antibacterial effectiveness of calcium hydroxide in disinfecting dentinal tubules and in the agar diffusion test. Haapasalo and Orstavik (7) reported that a calcium hydroxide paste (Calasept, Swedia, Knivsta, Sweden) failed to eliminate, even superficially, *Enterococcus faecalis* in the tubules. Safavi et al. (8) observed that *Enterococcus faecium* remained viable in dentinal tubules after relatively extended periods of calcium hydroxide/saline solution treatment. Siqueira and Uzeda (9) verified that calcium hydroxide/saline solution paste was ineffective in eliminating *E. faecalis* and *Fusobacterium nucleatum* from the tubules even after 1 wk of exposure. In contrast, a calcium hydroxide/CPMC/glycerin paste effectively killed bacteria in the tubules after a 1-h period of exposure, except for *E. faecalis* that required 1-day of exposure. Leonardo et al. (10) have reported the efficacy of calcium hydroxide/CPMC paste as an antibacterial dressing. Results of the agar diffusion test revealed that calcium hydroxide mixed with distilled water, saline, methylcellulose, or glycerin has little or no antibacterial effects (9, 11–13), which probably occurred because of the low diffusibility and solubility of the paste, and the buffering ability of the medium. Conversely, calcium hydroxide associated with CPMC showed pronounced antibacterial activity (11, 13).

Therefore, the purpose of this study was to evaluate the influence of different vehicles on the antibacterial effects of calcium hydroxide using a broth dilution test.

MATERIALS AND METHODS

Calcium hydroxide powder was mixed with the following vehicles: (a) 0.85% saline solution; (b) glycerin; or (c) CPMC and glycerin. The calcium hydroxide mixtures were prepared to a creamy consistency. In paste (c), glycerin and CPMC were used in a 1:1 ratio (vol:vol).

Antibacterial effects of the calcium hydroxide pastes were evaluated against two obligate and two facultative anaerobic bacteria. The obligate anaerobic strains were *Porphyromonas endodontalis* (BN 11a-f, kindly supplied by Dr. G. Sundqvist) and *Prevotella intermedia* (ATCC 25611). The facultative strains were *Streptococcus sanguis* (ATCC 10556) and *E. faecalis* (ATCC 29212). These bacterial strains were grown in prereduced anaerobically sterilized brain heart infusion broth supplemented with hemin and menadione (BHI-PRAS). Inoculum was prepared in this culture medium by adjusting to the turbidity of a 0.5 McFarland $BaSO_4$ standard.

For the test, 1 ml of the prepared calcium hydroxide pastes was placed at the bottom of wells of 24-well cell culture plates (Corning Glass Works, Corning, NY). Wells containing 1 ml of 0.85% saline solution served as the control. Each paste was placed into two wells for each bacteria tested. Afterward, 1 ml of culture of the

TABLE 1. Antibacterial effects of calcium hydroxide associated with three vehicles

Bacterial Strains	Control (Saline)					CH + Saline Solution					CH + CPMC + Glycerin					CH + Glycerin				
	5 Min	30 Min	1 H	1 Day	3 Days	5 Min	30 Min	1 H	1 Day	3 Days	5 Min	30 Min	1 H	1 Day	3 Days	5 Min	30 Min	1 H	1 Day	3 Days
P. endodontalis	+	+	+	+	+	+	+	+	−	−	+	+	−	−	−	+	+	+	−	−
P. intermedia	+	+	+	+	+	+	+	+	−	−	+	+	−	−	−	+	+	+	−	−
E. faecalis	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−	+	+	+	−	−
S. sanguis	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−	+	+	+	+	−

CH, calcium hydroxide; +, growth; −, no growth.

bacteria to be tested was placed into the wells containing calcium hydroxide pastes or saline.

The cell culture plates were then placed into anaerobic jars, in which the anaerobic conditions were obtained by the evacuation-replacement procedure, as follows: the air in the jar was removed by means of a vacuum pump and replaced with a mixture containing 10% H_2 and 10% CO_2 in nitrogen. The jars were incubated at 37°C for different periods of time. After time intervals of 5 min, 30 min, 1 h, 1 day, and 3 days (following the addition of fresh media to the wells), aliquots of 0.1 ml were taken from each well and transferred to tubes containing 5 ml of fresh BHI-PRAS. All of the tubes were vortexed and then incubated at 37°C for 1 wk.

Growth of the bacteria was observed at the end of the period of incubation by the presence of turbidity in the tubes. Whenever turbidity was determined, the purity of the cultures was checked by Gram-staining and morphology of colonies grown onto blood agar plates.

RESULTS

From the data shown in Table 1, it can be observed that all pastes were effective in killing the bacteria tested, but this effect varied with time. The calcium hydroxide/CPMC/glycerin paste was the most effective against the four bacterial strains tested. Bacteria survived in saline (control group) until the end of the experiment (i.e. 3 days).

DISCUSSION

The dilution broth susceptibility test is an effective method to evaluate the bactericidal properties of antimicrobial agents (14), allowing direct contact in solution between bacterial cells and the agent to be tested. This is critical when one is evaluating the antibacterial activity of calcium hydroxide, which has a low solubility and diffusibility.

The results are in agreement with others with regard to the antibacterial effectiveness of calcium hydroxide (4, 5). However, the time required to kill bacterial cells was different when compared with other studies, which may have been because of the differences in methodologies, inoculum size and age, culture medium, and bacterial strains.

Calcium hydroxide kills bacteria because of the effects of hydroxyl ions. These ions are extremely reactive, combining rapidly with lipids, proteins, and nucleic acids. They cause lipid peroxidation, increasing the bacterial membrane permeability; protein denaturation, inactivating enzymes; and DNA damage (3, 15). These phenomena can cause bacterial death.

Nevertheless, killing of bacteria by calcium hydroxide will depend on the availability of hydroxyl ions in solution, which is higher where the paste is applied. Where calcium hydroxide needs to diffuse to tissues and the hydroxyl concentration is decreased as result of the action of buffering systems (bicarbonate and phosphate), acids, proteins, and CO_2, its antibacterial effectiveness may be reduced or impeded.

This study demonstrated that the calcium hydroxide/CPMC/glycerin paste rapidly kill bacteria. The other pastes also killed bacteria, but required more time. This finding clearly indicates that the CPMC increased the antibacterial effectiveness of the calcium hydroxide paste. In addition, this mixture possesses a higher radius of action, eliminating bacteria located in regions more distant from the site where the paste was applied (9). Therefore, we believe that CPMC cannot be considered a vehicle to calcium hydroxide, but as an additional medication.

Studies have revealed that the association of calcium hydroxide with CPMC is biocompatible (10, 16, 17). Probably, the denaturing effect of calcium hydroxide on connective tissue may prevent or reduce the perirradicular tissue penetration of CPMC, reducing its cytotoxicity.

Based on data from this study, calcium hydroxide/CPMC/glycerin paste seemed to be the most effective antibacterial intracanal medication tested. Further in vivo studies may address the higher antibacterial effectiveness of this paste as an intracanal medication.

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The Way It Was

In 1905, Alfred Einhorn—a scientist working for the German chemical company Hoechst—isolated the procaine molecule. One year earlier in 1904, another chemist, Friedrich Stolz (who worked for the same company), synthesized epinephrine, the first hormone to be created synthetically.

By combining the vasopressor epinephrine with the procaine molecule, the toxicity of procaine was reduced and its duration of action increased, while allowing smaller volumes of the drug to be effective for local anesthesia.

Thus, the combination of two independent discoveries yielded a product that proved to be the standard for local anesthesia for the first half of the 20th century.

Suggested by Joel Dunsky