Antibacterial efficacy of calcium hydroxide, iodine potassium iodide, betadine, and betadine scrub with and without surfactant against *E faecalis* in vitro

Nathan E. Baker, DMD,¹ Frederick R. Liewehr, DDS, MS,² Thomas B. Buxton, PhD,³ and Anthony P. Joyce, DDS,⁴ Fort Gordon, Ga

**US ARMY ENDODONTIC RESIDENCY PROGRAM AND DWIGHT D. EISENHOWER MEDICAL CENTER**

**Objective.** This study investigated the ability of endodontic irrigants and medicaments to eliminate *Enterococcus faecalis* from infected dentinal tubules, and whether their antimicrobial action was enhanced by surfactant.

**Study design.** For the study, 5-mm dentin disks were sectioned from bovine incisor roots and infected with *E faecalis*. Lumens were instrumented, and 1 of 7 medicaments (10% Ca(OH)₂, Betadine, or IKI, each with or without surfactant, or Betadine Scrub) was used to flush and fill each lumen. Positive controls received saline. Specimens were incubated for 15 minutes or 24 hours. Quantitative microbiology of the remaining bacteria was performed and groups were compared using a 1-way ANOVA.

**Results.** The addition of surfactant did not enhance the antibacterial action of any medicament. When used as a 24-hour medicament, Ca(OH)₂ consistently failed to eliminate *E faecalis*, whereas both Betadine Scrub and IKI rendered 90% of samples sterile. IKI was the only agent shown to consistently eliminate *E faecalis* in a 15-minute time frame.

**Conclusion.** Under the in vitro conditions of this study, IKI was able to eliminate *E faecalis* from bovine root dentin when used with a 15-minute contact time.


The endodontic community is currently divided over the issue of whether infected cases can be successfully treated in a single appointment.¹ Sjögren et al.² found a 94% 5-year success rate for nonsurgical root canal therapy if a negative culture was obtained at the time of obturation, versus a 68% success rate if the culture results were positive at obturation, which implies that elimination of viable microorganisms from the root canal system greatly improves the prognosis of therapy. The question appears to be whether it is possible to eradicate or sufficiently reduce the number of bacteria in the root canal system in a single visit.

When endodontic therapy is performed in multiple appointments, it is common practice to use an intracanal medicament between visits in an attempt to kill any residual bacteria.³ Calcium hydroxide (Ca(OH)₂) has been shown to eliminate most microorganisms when used as a 7-day dressing,⁴ and is by far the most often used intracanal medicament in the United States.³ Recent research, however, has demonstrated that the most commonly isolated bacteria in failed endodontic cases, *Enterococcus faecalis*,⁵,⁶ are able to survive in the dentinal tubules despite long periods of Ca(OH)₂ therapy.⁷,⁸
Attempts to increase the antibacterial efficacy of Ca(OH)$_2$ against *E faecalis* have included the addition of additional medicaments such as camphorated monochlorophenol (CMCP). Spángberg found CMCP and 2% iodine potassium iodide (IKI) to have equal and potent antimicrobial effects, but noted that CMCP was toxic, even when used at a concentration 80 times lower than 2% IKI. Behnen et al varied the water-to-powder ratio of Ca(OH)$_2$, and demonstrated that a thin 10% mix reduced *E faecalis* counts more and at greater intratubular depths than did a thick mix. However, they did not demonstrate total killing within the time frame of their study. Barbosa et al demonstrated that adding detergent to Ca(OH)$_2$ solutions reduced the contact time needed to kill a wide range of bacteria, including *E faecalis*. The authors suggested that the surfactant may accelerate and enhance the antimicrobial action of Ca(OH)$_2$ by lowering its surface tension and by modifying the selectivity of the bacterial cell membrane to calcium and hydroxyl ions.

Hancock et al suggested that IKI, an irrigant with demonstrated low tissue toxicity, could be used as an irrigant since IKI “appears to be more potent than sodium hypochlorite or chlorhexidine in eliminating *E faecalis*.” Safavi et al reported that IKI eliminated *Streptococcus faecium*, a closely related but distinct species, from infected tubules in 10 minutes, compared to Ca(OH)$_2$, which took 24 hours. Ørstavik and Haapasalo reported that IKI was able to penetrate the dentinal tubules to eliminate *S sanguis* at a depth greater than 1000 μm within 5 minutes. A clinical study by Peciuliene et al investigated the ability of single appointment chemomechanical preparation followed by a 5-minute irrigation with IKI to eliminate yeasts, enteric gram-negative rods, and *Enterococcus* species from the root canals of previously root-filled teeth with chronic apical periodontitis. Initial microbial sampling showed growth from 82.5% of the teeth, with *E faecalis* present in 64% of the culture-positive teeth, yet only 1 sample (2.5%) yielded growth following the final flush with IKI.

Betadine (povidone iodine 10%) (Purdue Frederick Company, Norwalk, Conn) and Betadine Scrub (a povidone-iodine solution that contains detergent) (Purdue Frederick Company, Norwalk, Conn) are leading antiseptics in US hospitals today. In 1976, Torneck advocated the use of povidone-iodine solution as an endodontic irrigant, citing its rapid antiseptic action against a wide range of microorganisms, low toxicity, hypoallergenicity, and decreased tendency to stain dentin compared to other iodine-containing antiseptics. Buck et al found that Betadine was capable of permeating throughout the endodontic tubules, but concluded that its effectiveness was dependent on the type of bacteria found therein. The effectiveness of IKI, Betadine, and Betadine Scrub against *E faecalis*-infected tubules has not been established.

The purpose of this study was to investigate the ability of Ca(OH)$_2$, Betadine, Betadine Scrub, and IKI to eliminate *E faecalis* from experimentally infected dentinal tubules, and to establish whether their antimicrobial action could be enhanced by the addition of a surfactant.

**MATERIALS AND METHODS**

The in vitro model for testing antibacterial activity of medicaments in dentinal tubules of bovine incisors developed by Haapasalo and Ørstavik was modified for use in this study. One hundred twenty-nine intact bovine central incisors, extracted from frozen jaws (Shapiro Packing Company, Augusta, Ga), were stored overnight in 5.25% sodium hypochlorite (NaOCl) for surface disinfection. The crowns and apical 5 mm were removed with a diamond saw (Isomet, Buehler LTD, Evanston, Ill) at slow speed (<100 rpm) with water cooling. The roots were prepared to cylindrical test specimens 5 mm in height with the pulpal lumen standardized to 2.5 mm with an ISO 025 round bur (Brasseler, Savannah, Ga). The smear layer was removed with 17% ethylenediaminetetraacetic acid (EDTA) in an ultrasonic bath for 4 minutes, followed by 5.25% NaOCl in an ultrasonic bath for 4 minutes to enable rapid bacterial penetration. The specimens were then placed in water and sterilized in a steam autoclave for 15 minutes at 121°C and 15 psi. The dentin cylinders were mounted in individual 22-mm diameter tissue wells (Corning Cell Wells, Corning...
Glass Works, Corning, NY) on a base of rope wax approximately 5 mm tall (Fig 1). The lumens of the dentin specimens were filled with approximately 25 μL of Brain Heart Infusion (BHI) broth (Difco, Detroit, Mich) containing 1.0 × 10^6 colony forming units (cfu) per mL (approximately 2.5 × 10^6 cfu/dentin disk) of E faecalis (ATCC 29212). Two milliliters of sterile saline were added to each tissue well (surrounding the wax base but out of contact with the dentin specimens) to ensure a humid environment while the specimens were incubated at 37°C in 5% CO₂ for 72 hours. Sterile BHI was added to each lumen every 24 hours as needed to compensate for any evaporation. At the end of the 72-hour incubation period, the bulk of the BHI broth was micropipetted from the lumens, and any remaining BHI was flushed from the specimens with a syringe using 2 mL of sterile saline. The dentin specimens were removed from the tissue wells and the lumens enlarged to 2.9 mm with a sterile slow speed ISO 029 round bur (representing clinical endodontic instrumentation). Once prepared, each specimen was immersed in sterile saline to remove any residual dentin shavings and patted dry with sterile gauze. The dentin cylinders were then remounted in sterile individual 22-mm diameter tissue wells on a base of rope wax approximately 5 mm tall.

In addition to a saline positive control group, there were 7 experimental agents tested in groups consisting of 10 specimens each for each experimental time frame tested:

- **Group 1**: Sterile saline controls
- **Group 2**: 10% Ca(OH)₂
- **Group 3**: 10% Ca(OH)₂ + sodium lauryl sulfate-containing Tween 20 surfactant
- **Group 4**: 2% IKI
- **Group 5**: 2% IKI + sodium lauryl sulfate-containing Tween 20 surfactant
- **Group 6**: povidone iodine (Betadine)
- **Group 7**: povidone iodine (Betadine) + sodium lauryl sulfate-containing Tween 20
- **Group 8**: Betadine Scrub (a proprietary povidone iodine solution with surfactant).

The 10 positive control specimens (Group 1) served to provide baseline data on bacterial growth over time and consisted of inoculated dentin specimens treated in the same manner as the 24-hour experimental groups except that they received sterile saline rather than a test medicament. A negative control group consisted of 10 specimens treated in the same manner as the positive control group with the exception that they were incubated with sterile, uninoculated BHI broth.

The solution for Group 2 was prepared by mixing 10 g Ca(OH)₂ USP per 100 mL sterile water. For group 3, 50 μL of Tween 20 (Sigma-Aldrich Corporation, St Louis, Mo) was added to each 10-mL increment of 10% Ca(OH)₂. The solution for Group 4 (2% IKI) was prepared by mixing 2 g of iodine (Sigma-Aldrich Corporation) in 4 g of potassium iodide (Sigma-Aldrich Corporation) and adding distilled water to a 100-mL volume. For group 5, 50 μL of Tween 20 was added to each 10-mL increment of 2% IKI. Solutions for Groups 6 and 8 were used as obtained from the manufacturer (Purdue Frederick Company, Norwalk, CT). Group 7 consisted of Betadine with the addition of 50 μL of Tween 20 added to each 10-mL increment.

One or two working times were tested for each test agent: 24 hours and 15 minutes. Each of the 7 medications was first tested for efficacy at 24 hours. Effective agents were further investigated at the 15-minute time frame.

Two milliliters of saline (in the control groups) or 2 mL of one of the various test solutions was used to flush and fill the lumen of each specimen. This solution was allowed to remain in the canal lumen for the prescribed time. Excess fluid was allowed to remain in the tissue well (surrounding the wax base but not contacting the specimen) to ensure a humid environment. All manipulations of the specimens were performed under a laminar flow hood (NUAIRE, Plymouth, Minn) to avoid contamination from outside organisms. The culture plates were labelled and the medicated cylinders within them were incubated under humid conditions at 37°C in 5% CO₂ for either 15 minutes or 24 hours.

After allowing a working time of 15 minutes or 24 hours, the experimental agent (or saline) was removed from the canal by flushing with 2 mL of sterile saline and patting the specimens dry with sterile gauze. The specimens were then frozen in a –70°C freezer in individualized, labelled containers, weighed, pulverized in liquid nitrogen using a mortar and pestle, placed in 2 mL PBS (phosphate-buffered saline), and vortexed for 5 minutes. Serial dilutions (1:10, 1:100, 1:1000, 1:10,000, and 1:100,000) were made, and 100-μL aliquots of the original suspension and each dilution were plated on BHI plates in triplicate. The BHI plates were incubated in 5% CO₂ at 37°C, and the number of bacterial colony-forming units was counted 24 hours later. The dilution, if any, that yielded a number between 30-300 bacterial colonies on the plates was used for data analysis.

Statistical analysis of the 24-hour data was performed with a Kruskal-Wallis 1-way analysis of variance (ANOVA) on ranks followed by an all-pairwise multiple comparison procedure (Student-Newman-Keuls method) (P < .05, n = 80). A 2-way ANOVA, followed by the Student-Newman-Keuls method (P < .05, n = 60) was used to compare the 15-minute data to the 24-hour data (across the 3 levels of medicaments tested at both time frames).
Fig 2. Number of surviving bacteria after 24-hour exposure to test medicaments.

Fig 3. Effect of exposure time on remaining bacteria (15 minutes vs 24 hours).

RESULTS

Negative controls were sterile. Mean bacterial counts, expressed in log_{10} cfu/disk, for each 24-hour medicament were as follows ("\(\sim\)" denotes significant differences): Saline (6.1 ± 0.2) > Ca(OH)\(_2\) (4.5 ± 0.7) = Ca(OH)\(_2\) + Tween (4.5 ± 0.4) > Betadine + Tween (3.0 ± 1.5) > Betadine Scrub (2.1 ± 1.5) > IKI (0.2 ± 0.6) = Betadine Scrub (0.3 ± 1.0) = IKI + Tween (0.2 ± 0.5) (Fig 2). The addition of Tween surfactant did not enhance the antibacterial action of any medicament. When used as 24-hour medicaments, both Betadine Scrub and the IKI preparations rendered 90% of the samples sterile. Betadine rendered only 10% of the samples sterile and Ca(OH)\(_2\) failed to eliminate E faecalis in any sample.

Mean bacterial counts, expressed in log_{10} cfu/disk, for the 3 agents tested as 15-minute irrigants were Betadine (3.3 ± 1.6) > Betadine Scrub (1.8 ± 1.7) > IKI (0.1 ± 0.4) (Fig 3). Both Betadine and Betadine Scrub were significantly less effective at 15 minutes than they were at 24 hours. IKI was the most effective 15-minute irrigant and was the only agent that was as efficacious at 15 minutes as at 24 hours of exposure time (Fig 4).

Results from the secondary investigation to determine whether there was a significant residual effect of the iodine-containing medicaments, expressed in log_{10} cfu/disk, were: Saline (6.5 ± 0.03) = Betadine (6.5 ± 0.8) = IKI (6.3 ± 0.2). As there was no significant difference between these groups, no residual effect of the iodine-containing medicaments could be confirmed.

DISCUSSION

Every iodine-containing agent tested performed significantly better than Ca(OH)\(_2\). In terms of percentage kill, using saline as a baseline, Ca(OH)\(_2\) eliminated roughly 94% of the bacteria. However, no E faecalis-infected specimen was rendered sterile by Ca(OH)\(_2\).
either with or without surfactant. In contrast, both Betadine Scrub and IKI rendered 9 of 10 samples bacteria-free within a 24-hour time frame. For comparison, the average number of remaining bacteria after a 24-hour treatment with IKI was 99.99% lower than the average number remaining after a 24-hour application of Ca(OH)$_2$.

Although the addition of the surfactant-containing Tween 20 failed to enhance any medicament’s action, Betadine Scrub’s proprietary surfactant did improve its antimicrobial action when compared to Betadine solution, suggesting that the interaction between surfactant type and the specific antimicrobial agent may be complex. This finding also raises the possibility that different types and/or concentrations of surfactants might yet be shown to be capable of enhancing the intratubular action of Ca(OH)$_2$ or the iodophors despite the failure of Tween 20 to do so, and should be the subject of further investigation.

Our results do not support those of Safavi et al., who reported a significantly lower rate of culture reversal when teeth whose canals were filled with Ca(OH)$_2$ were compared to those having a cotton pellet soaked with IKI placed in the pulp chamber. In the present study, Ca(OH)$_2$ and IKI were used in identical fashion, filling the lumen of the samples for the 24-hour period. This difference in study design and medicament application may explain the discrepancy in results between the 2 studies. In addition to the lack of intimate contact with microbes in the root canal and dentinal tubules, an IKI-impregnated cotton pellet placed in the chamber would lack the mechanical occlusive effect of Ca(OH)$_2$ placed in the root canal that Siqueira has noted may be its primary effect of preventing culture reversal.

Currently it is recommended that teeth with infected root canals be treated in multiple visits with placement of a germicidal intracanal medicament such as calcium hydroxide in an attempt to eradicate these pathogens and ensure a successful therapeutic result. Unfortunately, multiple visits entail considerable additional time and expense for both patient and dentist, and unrestored and often broken down teeth are subject to what has been seen experimentally as culture reversal—reinfection due to intervisit microleakage and to the reestablishment of the microbial ecology of the root canal by residual bacteria. Ideally, an agent could be identified that would avoid the necessity of placing an intracanal medicament between visits by sterilizing the root canal with a contact time of minutes rather than days, allowing immediate obturation and restoration of the tooth. The most stimulating potential use for the iodophores, therefore, may not be as intracanal medicaments but as irritants. Peciuliene et al. reported in a clinical study that instrumentation and NaOCl irrigation in conjunction with final IKI irrigation was able to render 95% of retreatment teeth culture-negative in a single appointment despite the majority of teeth being infected with E faecalis. In our study, Betadine and Betadine Scrub, when used as 15-minute irrigants, produced an average of $3.3 \pm 1.6$ and $1.8 \pm 1.7 \log_{10}$ remaining cfu/disk respectively, which compare favorably with the $3.0 \pm 0.9 \log_{10}$ remaining cfu/disk produced by full-strength NaOCl in our pilot study. IKI performed significantly better than either Betadine or Betadine Scrub, rendering 9 of 10 samples completely sterile within the 15-minute time frame. As with any antimicrobial, the effectiveness of IKI may vary across species.

Further research is indicated to explore the effectiveness of IKI as an irritant across the range of endodontic pathogens.

In conclusion, Betadine Scrub and IKI are both capable of eliminating E faecalis when used as 24-hour medicaments. More importantly, IKI has a very high probability of eliminating E faecalis when the contact time is as short as 15 minutes, which corresponds to the clinical contact time of an endodontic irrigant. Although extrapolation from this work to the clinical situation must be guarded due to the presence of a smear layer, sclerotic dentin, and other factors in the in vivo situation, further investigation is indicated to explore the potential of IKI to sterilize root canals when used as an endodontic irrigant.

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REFERENCES


Reprint requests:
Frederick R. Liewehr, DDS, MS
Department of Endodontics
VCU School of Dentistry
PO Box 980566
Richmond, VA 23298-0566
frliewehr@vcu.edu