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The *in vitro* Antibacterial Effect of Iodine-potassium Iodide and Calcium Hydroxide in Infected Dentinal Tubules in Different Time Intervals

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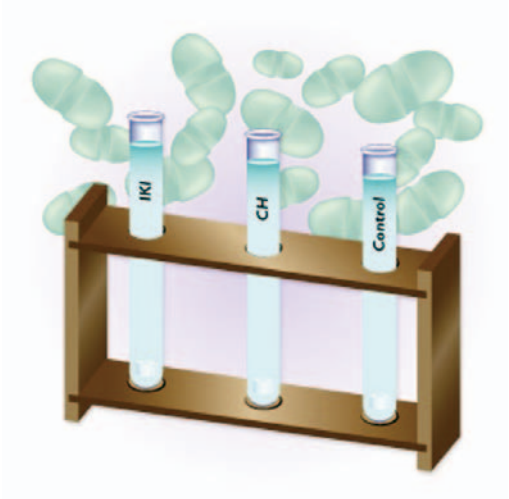

Abstract

Aim: The aim of this study was to evaluate the antibacterial effect of iodine-potassium iodide (IKI) and calcium hydroxide (CH) on dentinal tubules infected with *Enterococcus faecalis* (*E. faecalis*) at different time intervals.

Methods and Materials: Hollow cylinders of bovine root dentin (n=45) were infected and divided into three equal groups filled with either IKI or CH and a positive control. After placing each medicament in the infected cylinders for time periods of 10 minutes, 48 hours and 7 days, microbiological samples were analyzed. At the end of each period, four 100 μm thick inner dentin layers (400 μm thick from each specimen) were removed using dental burs of increasing diameters. Dentin powder was cultured on agar plates to quantitatively assess their infection, expressed in colony forming units (cfu).

Results: In all layers of the positive control group, heavy bacterial infection was observed. After 10 minutes, IKI reduced the amount of viable bacteria more efficiently than CH, whereas at later time intervals CH showed the best results.

Conclusion: For short periods of exposure, IKI has a more efficient antibacterial effect in the dentinal tubules than CH but CH performs better after longer durations of exposure.

Clinical Significance: This research indicates the use of IKI is a better choice for disinfecting the root canal than CH if only a short duration of exposure is used because of its more efficient antibacterial effect. However, if a longer exposure time is used, then CH is a better choice because of its better disinfecting effect over time.

Keywords: Iodine-potassium iodide, IKI, calcium hydroxide, CH, dentinal tubules, disinfection

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Introduction

The correlation between bacteria and endodontic lesions has been reported[1,2] as well as the crucial role bacteria plays in endodontic treatment failure.[3] Mechanical removal of bacteria and organic tissue from the root canal is an important course of action for the success of endodontic treatment, but it is mostly limited to the main canal.[4] Irregular spaces, such as root canal ramifications, isthmuses, apical deltas, and infected dentinal tubules, remain inaccessible.[5] The surviving bacteria could rapidly multiply and even return to their initial level between appointments when canals remain empty.[6] Intracanal medication is essential to completely disinfect the root canal space.[7]

Calcium hydroxide (CH), an effective intracanal medication, is considered the drug of choice by many clinicians[8] and is an effective antibacterial drug when mixed with other antibacterial agents.[9] In primary apical periodontitis (a primary endodontic infection) the microbial flora is sensitive to this medicament,[7] but in re-treatment situations it is not uncommon to find *Enterococcus faecalis* (*E. faecalis*) in the microbial flora.[10-12] *E. faecalis* is highly resistant to CH.[13,14] The low solubility and selective penetration of CH into dentinal tubules limits its antibacterial effectiveness as an intracanal medicament.[15,16] In an *in vitro* study[17] CH mixed with electrophoretically-activated copper demonstrated a strong antibacterial effect on infected dentinal tubules extending as deep as 500 μm.

Iodine-potassium iodide (IKI) is a biocompatible antibacterial agent.[18] In an *in vivo* study[19] teeth with necrotic pulps and periapical lesions that were pretreated with IKI before placing CH did not achieve an increase in the overall

antimicrobial effect. However, the strategy did reduce the frequency of persisting strains of *E. faecalis* resistant to CH. Conversely, IKI alone demonstrated a strong antibacterial effect in dentinal tubules infected with *E. faecalis* within 10 minutes of application.[20]

The purpose of the present study was to evaluate and compare the antibacterial effect of IKI and CH on infected dentinal tubules at different time intervals.

Methods and Materials

Preparation of Specimens

The model to assess infection of dentinal tubules of the root canal *in vitro* was originally described by Haapasalo and Ørstavik.[15] In the present study a modification suggested by Assouline et al.[21] was used. Extracted, non-carious bovine incisors were maintained in 0.5% NaOCl overnight for surface disinfection. The apical 5 mm and the occlusal two-thirds of the crown were cut with a rotary diamond saw (Isomet Plus precision saw,

Buehler, Cave Bluff, IL, USA) at 1000 rpm under cool water and discarded so the middle third of the bovine incisor was retained for further experiment. Cementum was removed with wet polishing paper in a variable speed grinder-polisher (Ecomet 3 Buehler, Cave Bluff, IL, USA), which resulted in a hollow dentin cylinder with a 6 mm external diameter. Cylinders were cut into 4 mm thick slices with the diamond saw. The 4 mm canal blocks were uniformly enlarged with an ISO 023 round bur using a slow-speed handpiece. During preparation, teeth and dentin slices were preserved in vials containing tap water to prevent dehydration. Organic and inorganic debris, including the smear layer, were removed by placing specimens in an ultrasonic bath with 17% EDTA (Sigma, Holon, Israel) for 5 minutes followed by 0.5% NaOCl for another 5 minutes.

Specimens were sterilized in their vials in an autoclave for 30 minutes at 121°C. Five specimens serving as negative control were incubated in growth medium at 37°C for 48 hours to confirm sterility.

Inoculation

Overall, 45 sterile specimens were randomly divided into three equal groups (n=15). Specimens from each group were placed into test tubes containing 50 ml of brain heart infusion broth (BHI) (Difco, Detroit, MI, USA) and inoculated with the test microorganism. The medium was changed every two days for 21 days. The inoculum consisted of BHI broth with *E. faecalis*, a common isolate from infected root canals[2] used in numerous studies of antibacterial properties because of its relative resistance.[15] The present study used a clinical isolate of *E. faecalis* T2 (Tel Aviv University Stock, Tel Aviv, Israel), which is resistant to 2 mg/ml streptomycin.[22] During the study streptomycin sulfate (Sigma, Holon, Israel), at a concentration of 0.5 mg/ml, was added to all growth media to prevent and overcome possible contamination in the experimental setup.

Medication

Intracanal medicaments examined were IKI 2/4% (Sigma-Aldrich Co. St. Louis, MO, USA) (2% iodine as the main active ingredient, dissolved in a 4% aqueous solution of potassium iodide because of its poor solubility in water) and CH in an aqueous paste (Dental Therapeutics AB, Gillevagen, Sweden).

Specimens were randomly distributed into three equal groups as follows:

- Group A (n=15) - Canals were medicated with IKI by placing a sterile cotton pellet soaked with 0.2 ml of IKI in the canal.
- Group B (n=15) - Canals were medicated with CH in aqueous paste, placed in the canal using a Hawe Composite-Gun tip No. 1914 (Hawe Neos Dental, Bioggio, Switzerland)
- Group C (n=15) - Served as positive control with no medication.

These groups were further divided into nine subgroups, each containing five specimens. Survival of the microorganisms was assessed at 10 minutes, 48 hours and 7-day intervals. At each time point, five specimens were tested. For the duration of this phase, test tubes were capped with aluminum foil and incubated at 37°C under humid conditions for the assigned time.

Sample Recovery

At the end of the experiment, the intracanal medicaments were removed using sterile paper points supplemented by curetting the intracanal lumen and outer aspects with a sterile curette in the CH group.

Dentinal samples were taken from each specimen with sterile round burs mounted in a handpiece and run at slow speed. The handpiece was mounted on a parallelometer to ensure centering of the bur in the canal. Specimens were held flat with sterile forceps during sampling and sequential burs were used in the following ISO sizes: 0.25, 0.27, 0.29, and 0.31 (Figure 1).

Each bur removed an approximate 100 μm thick layer of dentin from the inner "canal" surface. Dentin particles of all five specimens in the subgroup were collected into 20 separate test tubes containing 1 ml BHI broth with streptomycin. The particles were matched by time and depth totaling 20 samples (four different bars sampling five specimens). Tubes were vigorously mixed for 1 minute using a Maxi Mix 2 vortex (Thermolyne, Dubuque, IA, USA). The contents of each test tube diluted serially (100 μl were transferred to an Eppendorf tube containing 900 μl of sterile

Figure 1. Schematic drawing which illustrates the procedure used to collect dentin particles. Use of successively larger burs removed approximately 100μm of dentin at each time interval into a tube containing BHI broth.

saline). The dilution (1:10) was repeated four times resulting in a dilution of 1:10,000. Triplicate samples of 0.01 ml were spread on agar plates incubated for 48 hours at 37°C. Growing colonies on the agar were counted and recorded as colony forming units (cfu).

Statistic Analysis

An analysis of variance (ANOVA) with repeated measures was used to compare the antibacterial effect of IKI and CH at different time intervals. Data were collected and analyzed using the SPSS 10.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

The cfu count represents an estimate of the quantity of viable bacteria present in dentinal tubules at a given depth because the turbidity was considered indicative of the amount of infection.[21] Since the quantity of cfu obtained was high the numbers were transformed into values of log (cfu). Figures 2-4 and Table 1 indicate the average log count (of cfu) obtained after applying the medication according to depth (in µm) at the different time intervals.

No bacterial growth was found in the negative control specimens. In the positive controls heavy bacterial infection was observed at the layer closest to the lumen, with decreasing severity from layer to layer up to the deepest one tested (300-400 μm).

IKI applied for 10 minutes (Table 1 and Figure 2) significantly reduced the bacterial count in all dentin layers ($p<0.0001$) compared to CH. This mode of action significantly reduced the number of viable bacteria at all tested depths ($p<0.0001$).

CH was significantly more effective ($p<0.0001$) than IKI in the first two layers (up to 200 μm) following exposure to the material for 48 hours (Table 1 and Figure 3). There was no statistical significance between IKI and CH in reducing viable bacteria compared with the positive controls in 200-400 μm.

After application for 7 days (Table 1 and Figure 4), CH was statistically more efficient than IKI in all dentin layers ($p<0.001$).

Discussion

The *in vitro* model for dentinal tubule infection described by Haapasalo and Ørstavik[15] is effective for detecting viable bacteria at various depths of dentinal tubules.[23,24] The ability to evaluate the antibacterial efficacy of volatile materials, such as IKI,[17] using this model offers more advantages than other *in vitro* models. In the initial model only information regarding bacterial presence is shown. However, in the present study the model was modified, i.e., inoculating the bacteria in agar plates and counting the cfu, which resulted in a quantitative measure of vital bacteria in various dentin depths.[21]

The duration of application affected the antibacterial properties of the materials. IKI had a fast and short effect, reducing the amount of bacteria within 10 minutes, while CH remained for 7 days until effective. These findings agree with previous studies, which show CH has a lasting antibacterial activity in the root canal space due to its high pH.[16,25] However, CH has poor solubility and its bactericidal effect in the presence of dentin is unsatisfactory.[15,26] IKI or electrophoretically-activated copper improves efficacy in bovine dentinal tubules.[17] However, since copper is toxic with side effects, e.g., tooth coloring, other medicaments are required.

Table 1. Average log of cfu obtained from each dentin layer after applying medicaments for 10 minutes, 48 hours, and 7 days.

Layer	IKI	CH	Control
10 min			
1	1.27 ± 1.16	4.25 ± 0.61	6.73 ± 0.5
2	2.27 ± 1.26	4.56 ± 0.25	6.19 ± 0.13
3	3.08 ± 0.51	4.6 ± 0.22	5.68 ± 0.2
4	3.28 ± 0.79	4.72 ± 0.33	5.09 ± 0.9
48 h			
1	4.26 ± 0.14	1.33 ± 1.23	6.29 ± 0.21
2	4.59 ± 0.4	3.26 ± 1.83	5.71 ± 0.08
3	4.39 ± 0.44	4.48 ± 0.51	4.83 ± 0.09
4	4.32 ± 0.49	4.15 ± 0.93	4.45 ± 0.25
7 days			
1	3.29 ± 0.67	0 ± 0	5.26 ± 0.56
2	3.52 ± 0.38	0.37 ± 0.34	4.61 ± 0.45
3	3.83 ± 0.4	3.24 ± 0.63	4.32 ± 0.34
4	3.96 ± 0.28	3.25 ± 0.22	4.03 ± 0.51

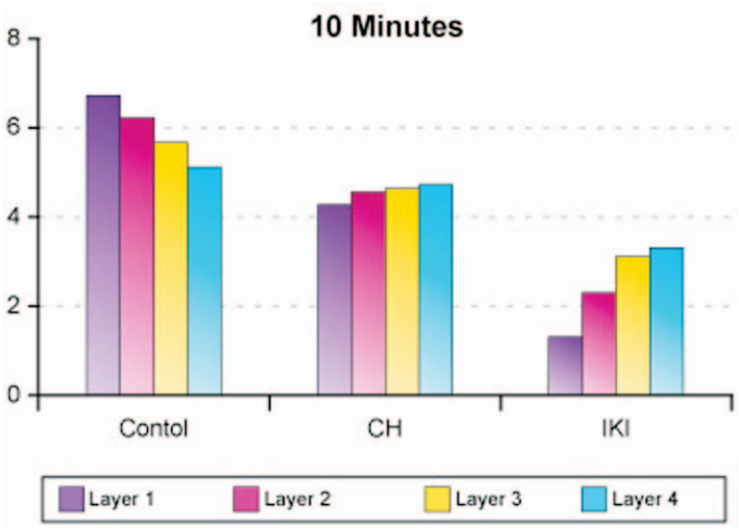

Figure 2. Average log of cfu obtained from each dentin layer after applying test medicament for 10 minutes.

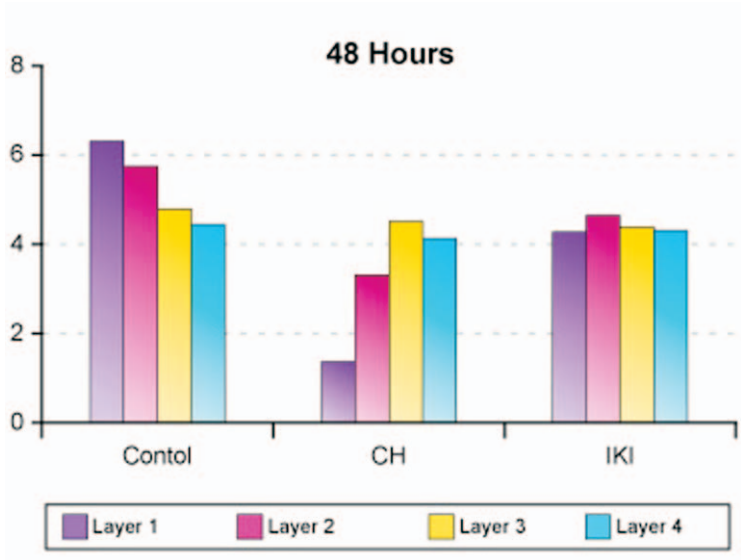

Figure 3. Average log of cfu obtained from each dentin layer after applying test medicament for 48 hours.

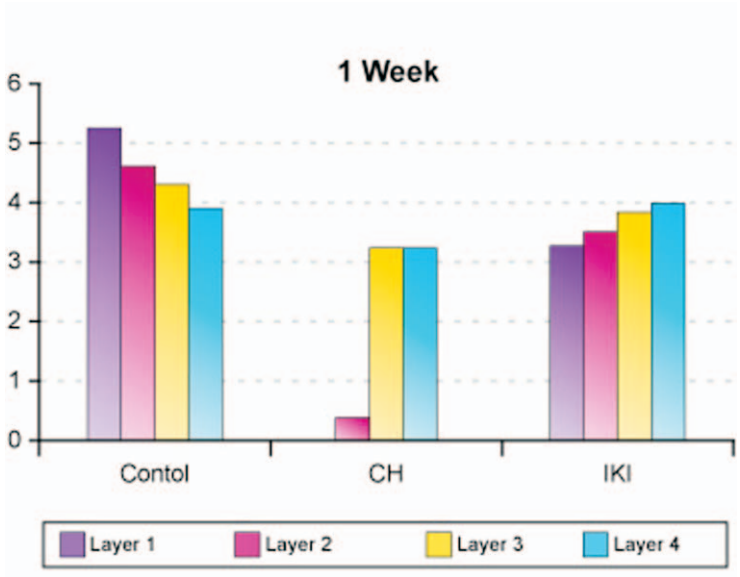

Figure 4. Average log of cfu obtained from each dentin layer after applying test medicament for 7 days.

IKI, a well-known antibacterial medicament, exhibits a long-distance bactericidal effect *in vitro* because of its evaporation and sublimation.[27] Compared with volatile medicaments, such as CMCP or formocresol, IKI is biocompatible.[28] It is prepared as 2% iodine dissolved in a 4% potassium iodide (w/v) (I_2/KI) solution in distilled water and placed in the root canal with a cotton pellet where it evaporates into the surrounding area. IKI is inactive in the presence of dentin powder *in vitro*.[26] IKI mixed with CH does not significantly alter the pH suspension or change the inhibition zone as shown with the agar diffusion test.[29]

Another contemporary intracanal medicament is chlorhexidine. A strong antibacterial effect has been shown in disinfecting dentinal tubules up to 500 μm when used in a slow release device. However, chlorhexidine has limited value in dissolving organic tissues from the root canal making it less favorable than CH as the drug of choice in endodontics.[30]

Root canal therapy with CH application as an intracanal medicament for at least 6 months is one of the possible protocols for treating inflammatory root resorption.[31,32] The rationale behind this is to disinfect the dentinal tubules. Since CH has low solubility, time is needed for it to penetrate and disinfect the dentinal tubules. The use of IKI for a short period followed by CH may reduce this time significantly as well as reduce the incidence of coronal root fractures during this period.[32]

In the present study, when medicaments were placed in the "canal" for 10 minutes, IKI had stronger antibacterial properties than the other groups assessed in all dentin depths. For longer applications (48 hours and 7 days), CH was more efficient. In disinfecting the dentinal tubules IKI was expected to be more efficient than CH. However, the opposite was shown. This could have been due to the escaping vapors of the medicament around the aluminum foil caps during incubation. The results might have been different if the caps were sealed.

The dentinal infection model represents an ideal situation in which the root canal is short (4 mm) and application and penetration of the medicament are simple. Further studies are required to test the effect of pretreatment with IKI prior to CH application as well as the penetrating ability and antibacterial activity of electrophoretically-activated CH in the apical third of root canals.

Conclusion

The best antibacterial effect into dentinal tubules was obtained by using CH for 7 days. However, for short periods (10 minutes), IKI showed the best results.

Clinical Significance

This research indicates the use of IKI is a better choice for disinfecting the root canal than CH if only a short duration of exposure is used because of its more efficient antibacterial effect. However, if a longer exposure time is used, then CH is a better choice because of its better disinfecting effect over time.

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