

THE ANTIMICROBIAL EFFECT OF IODINE-POTASSIUM IODIDE AFTER CLEANING AND SHAPING PROCEDURES IN MESIAL ROOT CANALS OF MANDIBULAR MOLARS

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ABSTRACT

The aim of this study was to evaluate the antibacterial activity of 2% iodine potassium iodide (IKI) used as a final rinse after the cleaning and shaping procedures in mesial root canals of mandibular molars infected with *Enterococcus faecalis*. Seventy two mandibular first molars were used. The root canals were infected with *Enterococcus faecalis* for 30 days. After the infection procedures, the root canals were cleaned and shaped by using the ProTaper rotary system and manual files. The teeth were randomly assigned to four experimental groups (N=18). In group 1, the root canals were irrigated with sterile distilled water (control). In group 2, the root canals were irrigated with 1% Sodium hypochlorite (NaOCl) during instrumentation. In group 3, root canals were irrigated with 1% NaOCl during instrumentation and a five minute final irrigation using 2 %

IKI. In group 4, the root canals were irrigated with 1% NaOCl during instrumentation and a 15 minutes final irrigation with 2 % IKI. Bacteria colony-forming units (CFU) from root canals were semi-quantified and the presence of negative cultures among the groups was compared using Fisher's test ($p < 0.05$). The order of effectiveness was: 1% NaOCl plus 2% IKI for 15 minutes (95%), 1% NaOCl plus 2% IKI for 5 minutes (44%), 1% NaOCl (17%) and sterile distilled water (0%). Fisher's exact test showed a significant difference among the groups ($p < 0.05$). It was concluded that under in vitro conditions, IKI was able to eliminate the *Enterococcus faecalis* from infected dentin significantly in a 15-minute time frame after the cleaning and shaping procedures.

Key words: dentin, dental materials, apical periodontitis, *Enterococcus faecalis*.

EFFECTO ANTIMICROBIANO DEL YODURO DE POTASIO YODADO SOBRE DENTINA INFECTADA

RESUMEN

El objetivo del presente trabajo fue determinar el efecto in vitro del yoduro de potasio yodado al 2% posterior a la preparación quimiomecánica en conductos radiculares infectados con *Enterococcus faecalis*. Para este estudio, se emplearon 72 primeras molares inferiores permanentes de humanos, los cuales fueron infectados con *Enterococcus faecalis* ATCC 29212. Los conductos fueron preparados mediante instrumentación rotatoria y distribuidos de manera aleatoria en cuatro grupos de acuerdo al irrigante empleado: Grupo 1, agua destilada estéril; Grupo 2, NaOCl al 1%; Grupo 3: NaOCl al 1% más IKI al 2% durante cinco minutos; y, Grupo 4: NaOCl al 1% más IKI al 2% durante 15 minutos. Se tomaron muestras pre y postoperatorias de los conductos y se realizó la semicuantificación microbiológica de las

unidades formadoras de colonias de las bacterias. Fue comparada la presencia de cultivos negativos en los grupos mediante el test de Fisher utilizando un nivel de significancia de $p < 0.05$. El orden de efectividad para la desinfección de los conductos radiculares de mayor a menor fue: NaOCl al 1% más IKI al 2% durante 15 minutos (95%), NaOCl al 1% más IKI al 2% durante 5 minutos (44%), NaOCl al 1% (17%) y agua destilada (0%). Se concluye, que bajo las condiciones in vitro de este estudio, el yoduro de potasio yodado empleado después de la instrumentación fue capaz de eliminar significativamente a la bacteria *Enterococcus faecalis* en un tiempo de 15 minutos.

Palabras clave: dentina, materiales dentales, periodontitis apical, *Enterococcus faecalis*.

INTRODUCTION

Bacterial invasion of the dental pulp results in inflammation, root canal infection and apical periodontitis^{1,2}. To eliminate the bacteria and necrotic pulp tissue, the root canal system must be cleaned and shaped using manual or nickel titanium rotary instruments in combination with sodium hypochlorite^{3,4}. In spite of the benefits of using sodium hypochlorite to control root

canal infection, many studies have shown that it is not possible to sterilize the root canal even using calcium hydroxide based root canal dressings⁵. *Enterococcus faecalis* has been reported to survive in calcium hydroxide treated root canals⁶ and under alkaline environments in laboratory studies⁷. Peciulienė et al. 2001⁸, reported that a final rinse of iodine-potassium iodide (IKI) after instrumentation and sodium hypochlorite

irrigation resulted in 95% culture-negative in retreatment cases. IKI presents low toxicity in comparison to formocresol⁹ and strong antibacterial properties have been reported when IKI was placed in *Enterococcus faecalis* infected dentin^{10,11}.

Dentin infection models are commonly used for evaluating the antibacterial activity of irrigants or root canal dressings. For standardization purposes, these models involve the use of dentin blocks or single root-end teeth^{12,13}. One advantage is the ability to evaluate the infection in samples with similar root canal diameters, improving statistical analyses. Anatomical irregularities are avoided and usually the antibacterial agent is in direct contact with the infected dentin. Irregularities of dental anatomy protect bacteria or biofilm from the action of irrigants or root canal dressings, making the total decontamination of necrotic root canals difficult¹⁴. Therefore, the use of more complex root canal anatomy seems prudent for *in vitro* evaluation of antibacterial protocols. The aim of this study was to investigate the ability of a nickel titanium rotary system used in conjunction with 1% sodium hypochlorite to eliminate the bacteria *Enterococcus faecalis* from experimentally infected mesial root canals of mandibular molars and to establish whether the antimicrobial procedure can be enhanced using a 5-minute or 15-minute final irrigation with 2% IKI.

MATERIAL AND METHODS

Specimen Preparation

Seventy two first mandibular molars were selected and stored in 10% formalin buffer. A root segment with a length of about 12 mm was prepared after the crown and the distal root were removed at the cemento-enamel junction. Type II and III mesial root canals as classified by Weine et al. 1988¹⁵ were used and no attempt was made to standardize the internal anatomy among the experimental groups. The teeth were washed with sterile water for 4 hours and stored in sterile water for one week to remove any residual chemical compound. Then the specimens were autoclaved for 20 min at 121°C and sterility was checked by incubating each specimen in 5mL of Brain Heart Infusion (BHI) broth at 37°C for 48 h.

Dentin infection with Enterococcus faecalis

The strain *Enterococcus faecalis* (ATCC 29212) was used in this study. To create the bacterial inoculum, isolated colonies of pure cultures of *Enterococcus faecalis* grown aerobically on Blood Agar plates were

suspended in 4.0 ml BHI. The cell suspension was adjusted to match the 0.5 McFarland turbidity standard. For dentin infection, all the teeth were transferred individually into 5 ml of BHI inoculated with 100µl of the *E. faecalis* suspension for 30 days. BHI was changed every 72 hours, and the purity of the broth was verified by gram test, catalase activity and growth in Bile Esculin Agar (HiMedia, Mumbai, India).

Root canal preparation

After the incubation period (30 days) the mesiolingual and mesiobuccal root canals were endodontically treated. For preoperative microbiological sample purposes, the pulp chamber was treated using 30% hydrogen peroxide for 3 minutes and 10% iodine tincture for 3 minutes⁶. The iodine solution was inactivated using a 5% sodium thiosulfate solution and a sterility control of the pulp chamber was taken using Thioglycolate Broth and sterile paper points. 100µl of each sample were incubated at 37°C for 48h in aerobic conditions in blood agar plates to confirm the sterility of the pulp chamber before the sample procedures.

The teeth were divided at random into 4 groups (N=18) according to the irrigation protocol used:

- G1: instrumentation using distilled water (control)
- G2: instrumentation using 1% sodium hypochlorite
- G3: instrumentation using 1% sodium hypochlorite and a final rinse of IKI 2% for 5 minutes.
- G4: instrumentation using 1% sodium hypochlorite and a final rinse of IKI 2% for 15 minutes.

The working length of each mesial canal was established by measuring the penetration of a 15 K-file (Flexofile, Dentsply Maillefer, Ballaigues, Switzerland) until it reached the apical foramen and then subtracting 1 mm. Both mesial canals were prepared with the ProTaper Rotary System (Dentsply-Maillefer). The handpiece was used at 250 rpm (X-Smart, Dentsply Maillefer). Instrumentation began with the use of the S1 and SX instruments up to the length corresponding to beginning of the root curvature. Instrumentation was finished with S1, S2, F1 and F2 instruments up to the working length. Additional enlargement was completed with nickel titanium hand files, with the mesial root canals to the diameter of a 35 K-file (Nitiflex-Dentsply Maillefer) using the balanced force technique, Roane et al. 1985¹⁶. In group 1 (G1), distilled water was used as control, 2mL for each file used. In groups 2 to 4, one percent sodium hypochlorite was used continuously during the root canal shaping, 2 mL for each file used;

0.5 mL of 17% EDTA (Biodinâmica, Brazil) was used at the end of the biomechanical preparation for 1 min and a final rinse of sodium hypochlorite was performed. In Groups 3 and 4 a final rinse of 2% iodine potassium iodide⁹ was performed for 5 minutes (G3) or 15 minutes (G4).

Pre and Post-operative samples were taken after the inactivation of the iodine compounds using 5% sodium thiosulfate for 5 minutes. Then, 100µl of thioglycolate broth was injected into the root canals and # 15 Hedstroem files were used in a filing motion in both mesial canals before the cleaning and shaping procedure (S1) and after the cleaning and shaping and final irrigation procedures (S2). Sterile paper points were introduced into the full working length of the canals for 60 seconds. The paper points were immediately transferred to sterile tubes containing 2 mL of prereduced thioglycolate broth that was used as the transport medium. Under a laminar flow 100µl of the undiluted sample were plated in bile esculin agar and incubated in aerobic conditions for 48h. The thioglycolate tubes with the paper points were incubated at 37°C for one week verifying the presence or absence of turbidity. The number of colony forming units (CFU) in S1 and S2 samples in the agar plates were semi-quantified according to Molander et al. 1999¹⁷ as:

0 (No growth)	: 0
1+ (Very sparse growth)	: 1 - 10 cfu
2+ (Sparse growth)	: 11 - 100 cfu
3+ (Moderate)	: 101 - 1000 cfu
4+ (Heavy)	: 1001 - 10000 cfu
5+ (Very heavy)	: > 10000 cfu

Fisher's exact test with a level of significance of $P < 0.05$ was used to determine the differences in the proportion of samples with positive or negative growth. All the analyses were performed using the Graphpad Software (La Jolla, Ca, USA).

RESULTS

Infection of all the teeth was confirmed in the S1 samples of groups 1-4, as very heavy (5+) showing the ability of the bacteria to infect the root canals. The semi-quantification scores of all the S2 samples are shown in Table 1. Post operative negative cultures were present in 3 samples of G2 (17%); 8 samples of G3 (44%) and 17 samples of G4 (95%). No negative culture was present in G1 when distilled water was used during the instrumentation procedures. Fisher's exact test shows significant difference among the groups ($p < 0.05$). Negative samples in the bile esculin agar showed absence of turbidity. The positive growth assessed by bile esculin agar plate was similar to that assessed by turbidity.

DISCUSSION

The elimination of bacteria from necrotic root canals is achieved by cleaning and shaping procedures and the use of antibacterial dressings. Several reports support the use of antibacterial solutions in an attempt to reduce the number of bacteria in root canals. The diameter of a 35.02 file was used at 1mm from the foramen after the use of the F2 instrument, since clinical antimicrobial efficacy using this diameter has been reported in comparison to a 30.04 diameter in mandibular molars when 1% sodium hypochlorite was used¹⁸.

As a limitation of the present study, it can be mentioned that no attempt was made to standardize the internal anatomy of the samples because this procedure is only plausible with the aid of microcomputed tomography¹⁹. It was assumed that the random distribution of the samples could reduce the bias caused by the irregular anatomy. It is known that the complexity of mesial root canal systems includes the isthmus, which may render bacteria difficult to retrieve in a microbiological sample¹⁴. However, the results

Table 1: Results of CFU semi-quantification of the experimental groups.

Semi-quantification	G1 (control)		G2		G3		G4	
	S1	S2	S1	S2	S1	S2	S1	S2
No growth	0	0	0	3	0	8	0	17
very sparse growth	0	0	0	3	0	10	0	1
Sparse growth	0	3	0	11	0	0	0	0
Moderate growth	0	15	0	1	0	0	0	0
Heavy growth	0	0	0	0	0	0	0	0
Very heavy growth	18	0	18	0	18	0	18	0

showed that a high probability of negative cultures can be found after instrumentation using 1% sodium hypochlorite and a final rinse of IKI 2% for 15 minutes in comparison to the other groups analyzed. Molander et al. 1999¹⁷, reported no beneficial antimicrobial efficacy after the use of 0.5% sodium hypochlorite and IKI as intracanal medication for 3-7 days. The authors explained the results basically by inactivation of this compound in presence of organic tissue or fluids in the root canal. Inactivation of the antimicrobial activity of several medications by dentin, bacteria and organic compounds has been previously demonstrated^{20,21}. In another

study, Kvist et al. 2004⁵ concluded that a final rinse with IKI had the same antibacterial efficacy than a calcium hydroxide based protocol.

As suggested by the results of this and others studies that showed a high antibacterial activity of 2% IKI in *Enterococcus faecalis* infected dentin, it seems prudent that a final irrigation protocol of IKI should be performed routinely before the use of calcium hydroxide based root canal dressings. Because of IKI diffuses easily in dentin and Ca(OH)₂ prevents regrowth of the bacteria by blocking nutrients or periapical fluids, a synergistic effect of these compounds should be studied in future clinical trials.

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