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The antimicrobial effect of apical box versus apical cone preparation using iodine potassium iodide as root canal dressing: A pilot study

MERETE MARKVART[1], GUNNAR DAHLÉN[2], CLAES-ERIK REIT[3] & LARS BJØRNDAL[1]

[1]*Department of Odontology, Cariology, Endodontics, Pediatric Dentistry and Clinical Genetics, School of Dentistry, Faculty of Health Sciences, University of Copenhagen, Denmark,* [2]*Department of Oral Microbiology, and* [3]*Department of Endodontology, Institute of Odontology, The Sahlgrenska Academy at University of Gothenburg, Sweden*

Abstract

Purpose. The purpose was to study the reduction of intra-canal microflora in premolars with apical periodontitis instrumented with either apical box or apical cone preparation and to provide measurements of intervention effects to allow proper power calculation in future clinical trials. ***Methods.*** Twenty-four patients were centrally randomized to apical box preparation (size #60) or cone preparation (apical size #25). The groups were comparable regarding the presence of primary caries and type of coronal restoration. In the course of canal preparation each tooth was irrigated with 2.5% NaOCl (12 ml). Lastly, the canals were filled with 17% EDTA (2 × 30 s) and 5% iodine potassium iodide (IKI) for 10 min. The canals were sampled for micro-organisms on four occasions: before instrumentation, after instrumentation, after application of IKI dressing and at the beginning of the second appointment 1 week later. Between the treatment sessions, the root canals were sealed with IRM cement. In the laboratory, culture techniques were used to measure microbial growth, which was classified as: none, very sparse, sparse, moderate, heavy or very heavy. ***Results.*** Initially, microbes were recovered in 88% of the teeth. Growth was classified as none in 35% of the teeth after instrumentation and in 50% after the application of IKI. Irrespective of the time of sampling, no significant difference in microbial growth reduction was observed between the two types of apical preparation. Based on the 1-week post-sampling, a power calculation revealed that over 900 patients are needed to show a difference of 9% between the two protocols tested. ***Conclusions.*** Future trials should be conducted using stringent protocols and as multi-centre trials for reaching the required information size.

Key Words: *apical enlargement, apical periodontitis, cone preparation, microbiological root canal sampling, RCT*

Introduction

Apical periodontitis most often is caused by intra-canal microbiota [1–3]. Thus, the treatment of teeth with apical periodontitis aims at complete eradication of the infecting micro-organisms. Several clinical studies have found that root canal preparation significantly reduces the number of micro-organisms [4–7], but infrequently results in a sterile root canal [8,9]. Therefore, instrumentation usually is combined with antimicrobial irrigation solutions and inter-appointment dressings. Such procedures may further reduce or eliminate the intra-canal microflora as measured by sampling and culturing [4,9,10].

Different opinions and schools of thought exist regarding the design of the root canal preparation. Apical box preparation is intended to remove (infected) dentine in the entire circumference of the apical portion of the root canal and to create an apical stop to facilitate obturation [11]. Cone preparation, introduced as the step-back technique, shapes the root canal and removes as little dentine apically as possible, and the focus is on the enlarged taper size, which should increase the effect of the antimicrobial irrigation and facilitate obturation [12,13]. Studies have shown that apical enlargement significantly reduces the number of bacteria in the root canal [4–7]. Typically, the same tooth has been used when comparing cone preparation and apical enlargement [14] or when investigating microbial growth reduction following apical enlargement alone [6,7]. It would appear that no randomized clinical trial

Correspondence: Merete Markvart, DDS, Research Assistant, Department of Odontology, Area of Cariology, Endodontics, Pediatric Dentistry and Clinical Genetics, School of Dentistry, Faculty of Health Sciences, University of Copenhagen, Nørre Allé 20, DK-2200 Copenhagen N, Denmark. Tel: +45 35 32 68 11. Fax: +45 35 32 65 05. E-mail: merma@sund.ku.dk

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(RCT) has compared teeth with either apical box or apical cone preparation, where both protocols are using IKI as a root canal dressing. An important issue when designing a RCT is to perform a power calculation, addressing the numbers needed to demonstrate a difference between two intervention groups, as shown within other areas [15–17]. We found it difficult to estimate in advance a realistic level of outcome between the two protocols and the expected effect on microbial growth based on root canal sampling. Therefore, it was decided to initiate a pilot study to provide initial measurements of the effects of intervention on microbial growth to provide a proper power calculation in future RCT, and to explore the hypothesis that the approach to apical preparation influences the antimicrobial effect of canal instrumentation, i.e. the large deep shape created by box preparation will be superior, in the antimicrobial respect, to the shape obtained by cone preparation. The specific aim of the study was to compare the reduction of the intra-canal microflora in teeth with necrotic pulps and apical periodontitis following the two apical preparation shapes, both using IKI as root canal dressing. The two protocols are evaluated by microbiological root canal sampling (MRS) and culturing.

Materials and methods

We undertook a centrally randomized, patient-blinded study with two parallel groups to compare the microbiological growth reduction after apical box preparation with that after cone preparation. For a pre-power calculation it was decided to enrol 25 consecutive patients. They were recruited at the Department of Odontology, Faculty of Health Science at the University of Copenhagen. Inclusion criteria were: 18–70 years old, premolars with necrotic pulp and apical periodontitis confirmed by radiography and it had to be possible to obtain an optimal working field. Exclusion criteria were: complicated root morphology (S-curvature), previous root filling and lack of informed consent from the patient.

The allocation sequence to apical box vs cone preparation was generated based on random tables. Concealed allocation was further achieved through central telephone randomization. One tooth was treated in each randomized patient and patients were unaware of the treatment assignment. Approval for the study was obtained from the Research ethics committee of Copenhagen and Frederiksberg in Denmark (KF 03 314020) and was registered with the Danish Data Protection Agency (J.nr. 2006-41-6838). The study was undertaken with the understanding and written consent of each participant and was conducted according to the Declaration of Helsinki 2008.

Clinical and microbiologic procedures

Participants were informed prior to treatment according to the ethical committee's guidelines and the teeth were treated with either apical box preparation or cone preparation, according to the randomization. In both protocols IKI was used as a root canal dressing. Primary caries and the type of restoration were recorded.

Clinical procedures before instrumentation

After removal of caries and deficient restorations the teeth were isolated with a rubber dam and disinfected with 30% hydrogen peroxide solution and 10% iodine. After inactivation with 5% sodium thiosulphate, microbiological samples were obtained from the working field with charcoal-impregnated cotton pellets. The pellets were transported to the laboratory in a transport medium (VMGA III) [18]. The initial MRS (S_1) was taken after obtaining access to the root canals. Root canals were filled with VMGA I and, after reaming the root canal walls with sterile Hedström files (SybronEndo Corporation, Orange, CA) using the technique of pumping and maximal removal (PMR) [18], the entire fluid volume of the root canal was absorbed with charcoal points left for 15 s in the canal and then transferred to the transport medium VMGA III. Working length was verified electronically with an apex locator (Raypex 5, VDW, Munich, Germany). If the tooth had two root canals, both were treated, but sampling was performed only from the facial root canal. In the course of root canal preparation, each canal was irrigated with 12 ml 2.5% NaOCl.

Box preparation

The coronal part of the canals was instrumented with PreRaCe. 08/35 (FKG Dentaire, La Chaux-de-Fonds, Switzerland) and RaCe. 02/20-40, taken to the working length. The apical part of the preparation was enlarged to #60 with Niti-flex (Dentsply Maillefer, Ballaigues, Switzerland) hand instruments using the concept suggested by Weiger et al. [19].

Cone preparation

The canals were instrumented with ProTaper® (Dentsply Maillefer, Ballaigues, Switzerland), SystemGT™ (Dentsply Maillefer) and Niti-flex hand instruments. The coronal instrumentation was done using the ProTaper SX and SystemGT. 06/20 and files were carried to length. The apical area was enlarged to the first file meeting apical resistance, to NiTi-flex #25 in 11 cases and to #30 in one case.

Clinical procedures after instrumentation

The post-instrumentation MRS (S_2) was obtained after inactivation of NaOCl with 5% sodium thiosulphate (30 s). Root canals were filled with VMGA I and the dentine was reamed within the entire canal using new sterile Hedström files. The total fluid volume of the root canal was absorbed using charcoal points left for 15 s in the canal and was then transferred to the transport medium (VMGA III). Following this, the S_2 canals were filled first with 17% EDTA (2×30 s) to remove the smear layer and then with 5% iodine potassium iodide solution (IKI) for 10 min. IKI was inactivated with 5% sodium thiosulphate (30 s). Post-medication samples (S_3) were obtained as described for S_2. The canals were left empty and the cavities were temporarily sealed with IRM cement (Dentsply DeTrey GmbH, Konstanz, Germany).

After 7 days the canals were re-entered. The teeth were isolated with a rubber dam and disinfected and sterility was checked according to the protocol described above. Canals were sampled for microorganisms (S_4) as described above. The root canals were then obturated with sealer (Apexit, Ivoclar Vivadent Inc., NY, United States) and gutta percha (cold lateral condensation technique).

Outcome measure

The primary outcome measure was based on *semi-quantification* of the number of bacteria present in the root canal before and after instrumentation, with the outcome variable 'total colony forming units (cfu)'.

Laboratory procedures

Immediately after sampling, the MRS was sent to the laboratory. Anaerobic incubation was performed with the hydrogen combustion method for 5–7 days on Brucella blood agar. Aerobic incubation was performed on blood agar for 2–3 days and in a semi-liquid medium (Hunton medium, 18) inoculation under a flow of oxygen-free gas. Dilutions were prepared to 10^2 to estimate the total viable count per millilitre, expressed as cfu. The bacterial species or groups were identified on the basis of colony morphology, gram staining characteristics and ability to grow in the absence or presence of oxygen. The microbiological growth was semi-quantified as very heavy, heavy, moderate, sparse and very sparse on the agar plates, corresponding approximately to >10 000, <10 000, <1 000, <100 and <10 cfu per 0.1 ml [9].

Species and genotype identification

The bacterial isolates were identified as described by Kvist et al. [9]. Isolated streptococcal strains were specified according to colony morphology on Mitis-Salivarius agar plates (MS Difco, Laboratories, Detroit, MI) and were primarily grouped into polysaccharide-producing (e.g. *Streptococcus mutans*, *Streptococcus salivarius* and *Streptococcus sanguinis*) and non-polysaccharide-producing streptococci (*Streptococcus anginosus* group including *Streptococcus intermedius* and *Streptococcus constellatus*). Enterococcci were identified based on bile esculin hydrolysis on an Enterococcosel agar plate (BBL, Becton, Dickinson and Company, Sparks, MD) and lactobacilli based on their growth on Rogosa SL agar.

After identification, three or more colonies of the *Lactobacillus* or enterococcal species isolated from the same root canal but from different sampling occasions were genotyped using random amplified polymorphic DNA (RAPD) with the primer KIT 01-01 to Kit 01-20 (US Biological, Swampscott, MA) according to Yoshino et al. [20]. The RAPD amplification reaction was modified and performed in a total volume of 25 µl, consisting of 2.5 µl of Taq (5 U/µl) Stoffel Fragment (Applied Biosystems, Foster, CA), 3.75 µl primer (100 pmol/µl) (US Biological Swampscott, MA), 5 µl $MgCl_2$ (25 mM), 6.75 µl MQ and 2.5 µl of DNA template (100 ng, 10 ng/µl). The DNA was further amplified and processed according to Yoshino et al. [20]. PCR products were separated through 7.5% polyacrylamide and visualized by silver staining.

Statistical methods

The chi-square test was used for comparisons between groups with a significance level set at 5%.

Results

We enrolled 24 patients in the trial, 13 premolars with one root and 11 premolars with two roots. There was an even distribution of large crown/resin restorations (7/8), small resin restorations (4/3) and deep carious lesions (1/1) between the two intervention groups.

Initially (S_1), microbes were recovered in 88% (21/24) of the teeth (Table I). However, a relatively high number of the samples showed low quantities of growth and in no case was growth classified as *very heavy*. After instrumentation (S_2), there was a greater frequency of no growth in teeth prepared with box preparation compared with teeth instrumented with cone preparation, 42% (5/12) vs 27% (3/11). The application of IKI (S_3) added a further antimicrobial effect to the procedures, but no significant difference in terms of microbial growth reduction was observed between the two types of apical preparation at any sampling point (Table I). Samples from the operation field showed growth in 5% (5/93). These samples disclosed different strains of bacteria from those found in the corresponding root canal.

Table I. Distribution of initial, post-instrumental, post-medication and pre-obturation samples according to growth.

	S_1		S_2†		S_3		S_4⋆	
Growth	Box	Cone	Box	Cone	Box	Cone	Box	Cone
None	2 (17%)	1 (8%)	5 (42%)	3 (27%)	4 (33%)	8 (67%)	4 (36%)	5 (45%)
Very sparse	2 (17%)	2 (17%)	1 (8%)	1 (9%)	4 (33%)	2 (17%)	—	—
Sparse	5 (42%)	1 (8%)	1 (8%)	4 (36%)	4 (33%)	1 (8%)	2 (18%)	—
Moderate	3 (25%)	6 (50%)	2 (17%)	3 (27%)	—	—	—	2 (18%)
Heavy	—	2 (17%)	3 (25%)	—	—	1 (8%)	5 (45%)	4 (36%)
Very heavy	—	—	—	—	—	—	—	—
Statistics	$p = 0.115$		$p = 0.852$		$p = 0.263$		$p = 0.877$	

S_1, initial sample; S_2, post-instrumentation sample; S_3, post-medication sample; S_4, pre-obturation sample.
†One sample was lost during laboratory procedures, ⋆ two patients did not return for S_4.

The samples were dominated by gram-positive and facultative bacterial species and, despite the diversity being low, the degree of complexity decreased from S_1 to S_3. There was a reduction of growth in the box preparation group, in which no samples showed moderate or heavy growth. In the cone preparation group, samples with heavy growth were present (Table II). Fifty-nine per cent (13/22) of all S_4 had growth. We found non-polysaccharide streptococci in 77% (10/13) of these. In two cases, where different sampling occasions revealed *Enterococcus faecalis* (one case) and *Lactobacillus casei* (one case), we found the same genotype of these two species on both occasions. The result of S_3 represents the entire treatment including the use of IKI as a root canal dressing. A power calculation demonstrated that, after S_3 and if the same trend in the results continued (34% difference in samples with no growth following preparation, in favour of a cone preparation), a sample size of at least 31 in each intervention group was required to demonstrate a statistically significant difference between the two protocols ($p < 0.05$). Using S_4 as a guide for power calculation the difference between the protocols was markedly dropped (10% difference in samples with no growth favouring af cone preparation) and a sample size of more than 450 patients in each intervention group was required to demonstrate a statistical difference between the protocols at a 2-sided alpha level of 5% (type 1 error) and 80% power (type 2 error of 20%).

Discussion

In this trial we examined the microbiological growth reduction after apical box and cone preparations using IKI as a root canal dressing. Initially, microbes

Table II. Classification of bacterial strains isolated from samples ($n = 93$).⋆

	S_1		S_2		S_3		S_4	
	Box	Cone	Box	Cone	Box	Cone	Box	Cone
Anaerobes								
Fusobacterium spp.	4	3	0	1	1	1	1	1
Prevotella spp.	5	2	0	1	2	0	0	0
Peptostreptoccus spp.	2	2	0	0	0	0	0	0
Gram positive anaerobic rods	2	0	0	1	1	0	0	0
Facultative								
Lactobacillus spp.	5	9	4	6	2	2	4	2
Streptococcus spp. (NPSP)	6	7	6	4	3	3	6	4
Streptococcus spp. (PSP)	2	2	1	1	1	1	0	0
Enterococcus spp.	1	1	1	1	1	0	1	1
Staphylococcus spp.	0	1	0	0	0	1	0	0
Candida spp.	0	0	1	0	0	0	1	0

⋆Three samples were lost during culturing.
NPSP, Non-polysaccaride-producing species; PSP, Polysaccaride-producing species; S_1, initial sample; S_2, post-instrumental sample; S_3, post-medication sample; S_4, pre-obturation sample.

were recovered in 88% of the teeth. Growth was classified as none in 35% of the teeth after instrumentation and in 50% after the application of IKI. No significant difference in terms of microbial growth reduction was observed between the two protocols based on the small size of the material (Table I).

It must be emphasized that the variations amongst teeth in terms of anatomy and composition of the microflora might significantly influence the microbiological outcome variables and hide possible differences between the two apical shapes. Teeth with one and two root canals were used. Only teeth with completely separate roots were included, as the risk of sampling contamination in teeth with two non-separated root canals is high, as previously discussed [7].

A critical problem in microbiologic sampling of the root canal is how to avoid false negative and false positive results. The risk of false positives caused by contamination during root canal preparation appears to be small in this study. All procedures were done after rubber dam application and chemical sterilization and a control sample from the working field was taken according to the Möller method [18]. Only a few working field samples with bacterial growth were found and they all presented different bacterial species than those recovered from the root canal.

The results in S_4 showed unexpectedly frequent representation of non-polysaccharide streptococci (*Streptococcus anginosus* group), sometimes growing 'heavily'. If we consider S_4 as a gold standard ('true sample') [21], we should expect to find some persistent bacteria that are non-growing in S_3 as a result of medicament inhibition [22,23]. Contamination by plaque and/or saliva and/or coronal leakage would account for bacteria in S_4. However, this is unlikely as we had no bacterial growth in the working field sample and contamination by saliva and plaque would have contained more diverse flora. We were able to identify some bacterial isolates to species and genotype level. In all cases the same strain (species) in earlier MRS (S_1 and S_2) from the same root canal was found (Table II) and it is plausible that the bacteria discovered in S_4 were persisting strains, giving a 'false negative' S_3.

From micro-CT studies it is apparent that a relatively large part of the root canal wall, between 30–80% of the surface area, remains unprepared after completed instrumentation [24–27]. Recently, a quantitative micro-CT variable was introduced that aims to measure the actual distance of dentine after preparation, the so-called DAP [28]. On the basis of two instrumentation methods focusing on apical box preparation, it was found that a mean peripheral distance of 50 μm was removed in the apical region after preparation. However, it is known that bacteria can invade much deeper into the dentinal tubules [29–31]. We also found that the amount of unprepared area was in the range of 25–30% in small root canals, whereas in larger root canals the unprepared area was twice that size, ~ 60% [28]. The large amount of unprepared areas in the root canal might be a reason for not finding any difference in microbiological growth reduction between the two investigated apical preparation shapes, both using IKI as a root canal dressing.

One of the aims of this study was to provide more information on intervention effect, which could be used as a basis for power calculation in future RCTs. Therefore, if the present results stand alone, we may run the risk of a type 2 statistical error. However, if the difference of 34% between the number of samples with no growth is correct, favouring a cone preparation, the applied power calculation showed that 31 patients are needed in each intervention group to reach a statistically significant difference between the treatments. However, as indicated by S_4 the results of S_3 might have 'false-negative' samples, therefore a much larger trial may be needed in each intervention group (>450) to reach a statically significant difference between the two protocols. This has also been an obstacle in other studies [32]. Therefore, upcoming clinical trials should be conducted using high-quality protocols so that they can be included, compared and analysed in future meta-analyses and eventually the so-called required information size may be reached, defining which intervention should be preferred [33,34]. It can be noted that available systematic reviews within other areas suffer from the fact that the number of high-quality RCTs is sparse, which makes interpretation more difficult [35]. Perhaps even more importantly, future RCTs should also realistically be conducted as large multicentre trials to obtain the number of patients needed.

Conclusion

No significant difference was found between the apical box vs cone preparation using IKI as a root canal dressing. A large trial is needed to demonstrate a significant difference in microbiological growth reduction favouring a cone box vs apical using IKI as a root canal dressing. To obtain the required information size, future RCTs should be performed using comparable high-quality protocols and, in particular, larger multi-centre trials may be the most realistic approach.

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