Comparison between the Antibacterial Efficacies of Three Root Canal Irrigating Solutions: Antibiotic Containing Irrigant, Chlorhexidine and Chlorhexidine + Cetrimide

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Abstract

Aim: The purpose of this study was to compare the antibacterial efficacy of three root canal irrigating solutions: An antibiotic containing irrigant, Chlorhexidine and Chlorhexidine + Cetrimide.

Methodology: 46 permanent maxillary central incisors were made into standardized segments and sterilized. 6 teeth were used to confirm sterility. The remaining 40 teeth were infected with *Enterococcus faecalis* for 28 days. They were irrigated with – Group A: Co-amoxiclav/ Citric Acid/Polysorbate-80 (CCP); Group B: 2% Chlorhexidine gluconate; Group C: 2% Chlorhexidine gluconate (CHX) and 0.2% Cetrimide (CTR); Group D: Saline- Positive Control. Samples were collected from within the canal; spread on 5% sheep blood agar and incubated at 37°C for 48 hours. Colony forming units (CFU) were enumerated and the values were statistically analyzed.

Results: The results showed CCP has better antibacterial activity than 2% CHX ($p<0.05$). But, 2% CHX + 0.2% CTR showed better antibacterial effect than both 2% CHX and CCP ($p<0.01$).

Conclusion:

Microbial inhibition potential of CCP observed in this study opens perspectives for its use as an intracanal irrigant. Synergistic action resulted from the mixture of Cetrimide and Chlorhexidine and its efficacy was greater than Chlorhexidine or CCP.

Key Words: Amoxicillin, Chlorhexidine, Cetrimide, Clavulanic Acid, Enterococcus faecalis, Root Canal Irrigants

Introduction

The endodontic microflora is typically a polymicrobial flora of Gram-negative and Gram-positive bacteria, dominated by obligate anaerobes. The bacteria associated with primary endodontic infections are mixed but are predominantly Gram-negative anaerobic rods, whereas the bacteria associated with secondary infection comprise only one or a few bacterial species-the most important of which is *Enterococcus faecalis*, facultative, anaerobic, Gram positive cocci [1]. They have been frequently isolated from cases of endodontic failures and its eradication from the root canal still remains a challenge. The use of antibacterial irrigants has been recommended as an important adjunct to mechanical instrumentation to eliminate or at least reduce the numbers of microorganisms.

Chlorhexidine gluconate, a cationic bisguanide has been used as an endodontic irrigant for its antibacterial effects, non toxicity and substantivity that results in antimicrobial effect for days to weeks preventing re-infection [2]. However, Chlorhexidine does have its drawbacks as it does not have tissue dissolution properties and tends to get inactivated in the presence of organic matter [3]. Also it is unable to eradicate biofilms efficiently according to studies by Dunavant et al. [4] and Arias Moliz et al. [5]. This points to the need to use Chlorhexidine together with another chemically compatible agent that enhances its efficacy against biofilms.

Cetrimide [cetyltrimethyl ammonium bromide]; a quaternary ammonium compound and a cationic detergent is active against many Gram-positive and Gram-negative bacteria and has the ability to eradicate biofilms [5].

Penicillins are the most frequently used antimicrobial agents. Due to their effectiveness, minimal toxicity and relatively low cost, penicillins constitute the first choice of antibiotics for odontogenic infections. Important classes of penicillins include penicillins G and V, which are highly active against Gram-positive cocci, and amoxicillin with an improved Gram-negative spectrum. β-lactamase inhibitors such as Clavulanic acid are used to extend the spectrum of penicillin against β-lactamase producing organisms. Amoxicillin alone and combined with Clavulanic acid has high antibacterial activity against *E. faecalis* [6]. Also, Pinheiro et al. tested, *in vitro*, the susceptibility to different antibiotics of *Enterococcus faecalis* isolates from canals of root filled teeth with periapical lesions and found that 100% of the isolates were susceptible to amoxicillin-clavulanic acid [7]. Jacinto et al. investigated the correlation between the composition of the bacterial flora isolated from infected root canals of teeth with apical periodontitis and tested the antibiotic susceptibility of five anaerobic bacteria most commonly found in the root canals of symptomatic teeth and observed that root canals from symptomatic teeth harbored more obligate anaerobes and a bigger number of bacterial species than the asymptomatic teeth. More than 70% of the bacterial isolates were strict anaerobes. Amoxicillin, amoxicillin + clavulanate and cephaclor were effective against all the strains tested [8].

The purpose of this study was to compare the antimicrobial activity of three groups of irrigants against *Enterococcus faecalis*; GroupA: Combination of Co-amoxiclav/ Citric acid/ Polysorbate-80 [CCP], Group B: 2% Chlorhexidine gluconate [CHX], Group C: 2% Chlorhexidine gluconate [CHX] + 0.2% Cetrimide [CTR].

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Methodology

Preparation of samples

Ethical approval for the study was obtained from the Medical Ethics Committee of Yenepoya Research Centre of Yenepoya University, Mangalore, India. 46 non-carious, single-rooted human maxillary incisors, which were extracted for periodontal reasons, were used in this study. Each tooth was radiographed to confirm the presence of a single canal. Calculus and tissue tags were removed using hand-scaling instruments. The teeth were soaked in 5.25% NaOCl [Reachem Laboratory Chemicals Pvt. Ltd, Chennai] for 30 minutes to remove residual loose tissue and debris from the root surface. The teeth were stored in gauze soaked with sterile saline till use to prevent dehydration. All the teeth were sectioned 14 mm from the apex with a carborundum disc using a low speed straight hand piece, so as to standardize root canals of all the teeth approximately to the same length. Pulpal remnants were removed using barbed broaches [SpiroColorinox, Dentsply Maillefer, Switzerland]

Instrumentation

An ISO #10 or #15 Kerr file [Dentsply/ Maillefer, Tulsa, Okla] was used to determine the working length by penetrating the apical foramen and pulling back into the clinically visible apical foramen. The root segments were mounted in wax bases for ease of instrumentation. All root canals were instrumented, using the stepback technique and the circumferential filing motion, to an International Organization for Standardization [ISO] #50. During cleaning and shaping, 1 mL of sterile distilled water was used after each instrument size. The segments were then removed from the wax bases.

Smear layer removal from the samples

The smear layer of the lumen was removed using 17% EDTA [B.N. Laboratories, Mangalore] in an ultrasonic bath for 4 minutes followed by 5.25% NaOCl in an ultrasonic bath for 4 minutes. Finally, the canals were flushed with 5 mL of distilled water to remove any debris and residual irrigants. The root canal apices were sealed with Type II GIC [GC Fuji, Tokyo, Japan] and coated with two coats of nail varnish to prevent bacterial leakage.

Sterilization of samples

Each tooth was sterilized in steam autoclave for 30 minutes at 15 psi (at 121°C). In order to evaluate the sterility; 6 teeth, used as negative controls, were incubated at 37°C in sterile Brain Heart Infusion broth [Himedia Laboratories Pvt. Ltd., Mumbai] for 48 hours to rule out any bacterial growth.

Inoculation

The bacterial strain used in the present study was *E. faecalis* [ATCC 29212]. An overnight pure culture of *E. faecalis* [ATCC 29212] in Trypticase soy broth [Himedia Laboratories Pvt. Ltd., Mumbai] was adjusted to optical density 0.5 turbidity on the McFarland scale [1.5×108 bacteria/mL]. The canals of the experimental teeth were cautiously inoculated using a micropipette with 2 µL of the freshly prepared suspension and sterile K-type files #15 were used to carry the bacterial suspension to the entire root canal length. The teeth were then incubated at 37°C for 28 days. Fresh culture medium was added to the canal at every two days after the initial inoculum.

Root canal irrigation

After incubation, the remaining 40 contaminated root canals were divided into four groups according to the irrigation regimen used. For this study; 1200 mg of Co-amoxiclav i.e. Amoxicillin 1000 mg and Clavulanic acid 200 mg [Clavam IV, 1200mg vial, Alkem Laboratories, India] was mixed with 60 mL of the liquid component of BioPure MTAD [Dentsply, Tulsa Dental, Tulsa, OK, USA] containing 4.25% Citric acid and 0.5% Polysorbate-80 [Tween-80].

Group A: [10 Teeth] Mixture of Co-amoxiclav, Citric acid, Polysorbate-80 [CCP]

Group B: [10 Teeth] 2 % Chlorhexidine [Hexidine, ICPA Health Products Ltd. India]

Group C: [10 Teeth] 2% Chlorhexidine and 0.2% Cetrimide [Merck Chemicals, India]

Group D: [10 Teeth] Positive Control- Saline

All teeth were handled with sterile gloves and sterile forceps to prevent contamination. A 26-gauge needle attached to an irrigant-containing, sterile 5 mL plastic syringe was inserted into the root canal until it reached the apex, and was then withdrawn approximately 1 mm so that it was no longer in contact with the root canal walls. 5 mL of irrigant was delivered into the canal over 5 minutes. All experimental teeth were then flushed with 15 mL sterile saline to prevent potential carry-over of the irrigants.

Sampling technique

A small amount of sterile saline was introduced into the canal, and an endodontic hand file was used in a filing motion to a level approximately 1 mm short of the root canal apex. The canal contents were aspirated with a 1-mL disposable tuberculin syringe and then placed into tubes containing 1 mL of sterile saline. Two paper points #45 were placed at the working length for 30 seconds each and also used to soak up the canal contents. Paper points were transferred to the same tubes containing 1 mL of saline and agitated in vortex for 1 minute. After 10-fold serial dilutions in saline, aliquots of 0.1 mL were plated onto blood agar plates and incubated at 37°C for 48 hours. The Colony-Forming Units [CFU] grown were counted and then transformed into actual counts based on the known dilution factors.

The obtained results were compared using Kruskal-Wallis and Mann Whitney- U test to detect the statistical difference between groups.

Results

Table 1 shows the comparison of mean CFU among four different groups by Kruskal Wallis ANOVA Test [$p<0.01$] which indicates that 2% CHX+ 0.2% CTR was the most effective at eliminating *E. faecalis* than the other irrigant solutions.

Table 1. *Comparison of mean cfu among four different groups by kruskalwallisanova test.*

Groups	N	MINIMUM	Maximum	Mean	Std. Deviation
GROUP A	10	1.08×10^3	1.640×10^3	1.315×10^3	193.06
GROUP B	10	1.260×10^3	1.8×10^3	1.560×10^3	171.01
GROUP C	10	0.8×10^3	1.420×10^3	1.086×10^3	177.90
GROUP D	10	1.22×10^3	1.18×10^4	6.838×10^4	28515.9

H= 31.115 [Kruskal Wallis Test]
p<0.01 [highly significant]

Table 2. *Pairwise comparison of four groups with respect to cfu using mannwhitney 'u' test.*

Groups	Z	p	Inference
GROUP A vs. GROUP B	-2.4946	0.0126**	sig
GROUP A vs. GROUP C	-2.3434	0.0191**	sig
GROUP A vs. GROUP D	-3.7796	0.0002*	hs
GROUP B vs. GROUP C	-3.5529	0.0004*	hs
GROUP B vs. GROUP D	-3.7796	0.0002*	hs
GROUP C vs. GROUP D	-3.7796	0.0002*	hs

**p<0.05, *p<0.01
Z = Mann Whitney 'U' Test
p = Probability
hs = highly significant
sig= significant

Table 2 indicates pair wise comparison of four groups with respect to CFU using Mann Whitney 'U' Test. Results indicate that in comparison with the Control; Group A, Group B, Group C showed p=0.002 which is highly significant. CCP showed a highly significant [p<0.01] difference in comparison with the control group. CCP showed better antimicrobial efficacy against *E. faecalis* as compared to 2% CHX alone and this difference was significant [p<0.05] But, the combination of 2% CHX+ 0.2% CTR showed better antimicrobial efficacy against *E. faecalis* as compared to CCP or 2% CHX.

Discussion

In this study, *Enterococcus faecalis* was chosen as the test organism because its prevalence has been a conspicuous finding in a high percentage of root-canal failures that has been attributed to its high resistance and its ability to survive as a single organism in monocultures [9-13]. It has been used in previous studies testing the efficacy of irrigant solutions [13,14]. The significant characteristics of *Enterococci* include their ability to grow in the range of 10°C-45°C and to survive for around 30 min at 60°C; and at high salt concentrations of 6.5% saline as well as at extremely alkaline pH of upto 11.5 [10,15] The characteristics of *E. faecalis*, which relate to its prevalence include its ability to endure nutritional deprivation, bind to dentin and invade dentinal tubules, compete with other bacteria, suppress lymphocytes, and produce toxins [9,16].

In the present study, the teeth were incubated with *E. faecalis* for 4 weeks to ensure adequate penetration of the bacteria into the dentinal tubules [17]. Moreover, the bacterial sample was collected using both paper points as well as tuberculin syringes after instrumentation of the canal space. Ando and Hoshino [18] demonstrated the presence of bacteria 500 to 2000 μm in tubules in teeth with heavily decayed crowns. Culturing of the dentin shavings and canal contents at a greater depth allowed determination of the efficacy to the test irrigants at penetrating and disinfecting deeper layers of dentine.

CHX is bactericidal at higher concentrations (1.8-2% and above) causing precipitation of bacterial cytoplasm and cell death [19,20] In this study CHX showed highly significant antibacterial activity. These results are in accordance with previous studies by Safavi et al. and Sassone et al. [19,20] that evaluated the efficacy of CHX against *E. faecalis*.

Cetrimide on the other hand has been shown to be effective in reducing biofilms when combined with irrigating solutions [5,21]. It produces little irritation and reduces the surface tension of liquids, thereby facilitating their entry into places of difficult access. In this study, 2% CHX+0.2% CTR demonstrated a highly significant antimicrobial activity against *E. faecalis* as compared to 2% CHX alone. Similar results were demonstrated in studies by Portenier et al. [22] and Arias Moliz et al. [5]. Both CHX and CTR target the cell wall of the organisms and may have a synergistic activity.

Recent findings suggested that bacteria in biofilms undergo a process of phenotypic diversification that decreases their susceptibility [23]; multiple cell types in single species biofilms might ensure population survival against one particular antimicrobial agent. Thus, treating biofilms with combinations of distinct antimicrobials might be an effective strategy to kill different cell types [24]. Furthermore; Cetrimide weakens the biofilm's cohesive forces, disrupting the extracellular polymeric substance matrix [25], responsible for biofilm mechanical stability and for binding it to some base or support. In this study Chlorhexidine and Cetrimide were not used alternatively but in a combination and proved to have significant antimicrobial property. To enhance the efficacy of the irrigants, the action on biofilms should involve the elimination of the extracellular polymeric substance matrix as well as the bacteria because this matrix could act as an additional source of nutrients and/or as a suitable surface for further cell growth. It would be interesting to study the effect of alternatively using CTR and CHX. Perhaps if CTR is applied first it would facilitate the destructuring of the

extracellular polymeric substance matrix. Thereafter, CHX might be able to act more directly on *E. faecalis*, possibly exerting its bactericidal potential to a greater degree.

Antibiotics are a valuable adjunctive to the materials available to health professionals for the management of bacterial infections. During endodontic treatment and when managing trauma to the teeth, antibiotics may be used systemically (orally and/or parenterally) or locally (i.e. intra-canal via irrigants and medicaments). Due to the potential risk of adverse effects following systemic application, and the ineffectiveness of systemic antibiotics in necrotic pulpless teeth and the periradicular tissues, the local application of antibiotics may be a more effective mode for delivery in endodontics.

Penicillins are the most frequently used antimicrobial agents. Due to their effectiveness, minimal toxicity and relatively low cost, penicillins constitute the first choice of antibiotics for odontogenic infections. Amoxicillin alone and combined with clavulanic acid has high antibacterial activity against *E.faecalis* [26]. Also, Co-amoxiclav is a commonly prescribed antibiotic during endodontic treatment [27]. Scukaite et al. evaluated the susceptibility of predominant endodontic pathogens isolated from teeth with symptomatic apical periodontitis to most commonly prescribed antibiotics and found that all tested micro-organisms were highly sensitive to amoxicillin and that amoxicillin is a suitable antibiotic for treatment of endodontic infection when conventional root canal treatment alone is insufficient [28]. However there is no previous study of Co-amoxiclav used in an irrigant. This study evaluated the antibacterial activity of Co-amoxiclav as a root canal irrigant. Citric acid and Polysorbate-80 were added to increase the efficiency of the combination in smear layer removal.

Polysorbate-80 has limited antibacterial activity, but it reduces the surface tension and interfacial tension of solutions thus increasing the adsorption and uptake of antibacterial solutions by bacterial cells thereby killing the cells at a faster rate. Thus addition of Polysorbate-80 enhances the antibacterial efficacy of these agents. The results of this study show that CCP has a highly significant antibacterial property and holds promise as an effective root canal irrigant.

Based on the above findings we can conclude that rather than using a single chemical as a root canal irrigant a combination of various antibacterial agents can be tried and tested against the endodontic microflora. However one must bear in mind that the antibacterial effectiveness of irrigants *ex vivo* may be quite different when compared to mixed cultures present in a dynamic biological system, as usually occurs *in vivo*. Thus the efficacy of the antibacterial agents in clinical conditions must be exercised with caution because of the obvious limitations of *ex vivo* studies.

Further research is required regarding the effect of this new mix of CCP on dentine and on single and multiple species biofilms.

Conclusion

The present study concludes that CCP showed better antimicrobial efficacy as compared to 2% CHX [$p<0.01$] and its microbial inhibition potential observed in this study opens perspectives for its use as an intracanal irrigant. Combinations of 2% CHX + 0.2% CTR showed better antibacterial effect than 2% CHX alone or CCP. So the present study also demonstrated that synergistic action resulted from the mixture of 2% CHX + 0.2% CTR.

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