Original Article

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Dentistry Section

Study Comparing the Effectiveness of Chlorhexidine, Calcium Hydroxide and Linezolid Based Medicaments Against *Enterococcus Faecalis*

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ABSTRACT

Purpose: This study evaluated the efficacy of 2% chlorhexidine (CX), calcium hydroxide (CH), Vitapex® (VP), linezolid (LZ), a combination of LZ with CH (LC) against *Enterococcus faecalis* (EF).

Study Design: EF strains were mixed with peptone water and the turbidity was adjusted to the McFarland's turbidity standard tube No: 0.5. The inoculum obtained was used to make lawn cultures on the agar plates. A total of 30 agar plates were prepared, such that each plate had five wells containing the five medicaments. The plates were incubated and evaluated for zones of inhibition after intervals of 24 hours and 72 hours. The results were statistically evaluated by paired t-test, ANOVA and Post-hoc analysis using Tukey's HSD.

Results: The difference between values of the zones of inhibition around various medicaments after 24 hours and 72 hours was found to be statistically significant. A comparison between the five groups after 24 hours or 72 hours showed that each group differed significantly from the rest of the groups.

Conclusions: LC had the greatest effectiveness against EF, followed by LZ, CX, VP and CH.

Keywords: Chlorhexidine, Linezolid, Calcium hydroxide, *Enterococcus faecalis*

INTRODUCTION

The quest for the best intra canal medicament for root canals has remained a struggle ever since endodontic literature has been written. Although, various medicaments exist, each has its advantages and disadvantages. *Enterococcus faecalis* (EF) has been found in asymptomatic and persistant root canal infections [1,2]. It has also been found in 77% of failed endodontic cases [2], and in 50% cases with chronic apical periodontitis [3]. Chlorhexidine (CX) has been known for its broad spectrum of action against gram positive and negative organisms. It has a unique ability to adsorb on to dental tissues and mucous membrane (Substantivity), while releasing itself gradually [4]. Calcium hydroxide (CH) has been popular as an intra canal medicament due to its additional action on gram negative bacteria [5,6]. Some authors have discussed its effectiveness in eliminating EF [7,8]. Combinations of CH with iodoform have been previously believed to enhance periapical bone regeneration simultaneous with resorption of excess material, with a success rate of 84% to 100% [9]. Vitapex® (VP), a combination of CH and iodoform, resorbs from apical tissues within a time span of a week to two months in primary teeth. It is radio-opaque, non-setting, easily inserted and retrieved [10].

Linezolid (LZ) has gained popularity due to its activity against gram positive organisms, including vancomycin resistant EF. It is an oxazolidine agent that acts by inhibiting the initiation of bacterial protein synthesis. It has a half life of four to six hours, gets 31% protein bound and has good CSF penetration [11]. The effectiveness of CH on EF, with or without iodoform, has been controversial. Moreover, the effectiveness of LZ, or a combination of LZ with CH (LC), on root canal related EF has not yet been reported. Hence, this study was conducted to evaluate the efficacy of CX, CH, VP, LZ and LC on EF at time intervals of 24 hours and 72 hours using the agar diffusion method. The null hypothesis assumed was that there would be no difference amongst the medicaments in their action against EF.

	n	At 24 hrs	At 72 hrs	t-value	p-value
LC	30	26.31 ± 0.597	27.13 ± 0.540	13.025	<0.001***
LZ	30	22.55 ± 0.560	22.78 ± 0.592	4.455	<0.001***
CX	30	15.37 ± 0.574	16.36 ± 0.545	56.512	<0.001***
VP	30	5.50 ± 0.426	1.57 ± 0.356	57.00	<0.001***
CH	30	0.68 ± 0.545	0.23 ± 0.264	7.072	<0.001***

[Table/Fig-1]: Comparison between groups after 24 and 72 hours using the paired t-test
*** $p < 0.001$; Highly significant

MATERIALS AND METHODS

The study was conducted in the Department of Conservative Dentistry and Endodontics, in association with the Departments of Microbiology, Pharmacology and Pharmaceutics. Agar plates were prepared on sterilized glass petri dishes and were left overnight at 37°C. EF strains were mixed with peptone water and the turbidity was adjusted to the McFarland's turbidity standard tube No: 0.5. The inoculum obtained was used to make lawn cultures on the agar plates using sterile cotton swabs. Following this, wells that were three millimeters in diameter and four millimeters in depth were punched on the agar plates. A total of 30 agar plates were prepared, such that each plate had five wells, into which each of the five medicaments were placed in no particular order. However, the medicament name was labeled under each well after each medicament was placed to enable a blind evaluation. The five medicament groups were:

	n	Range	Mean	SD	SEm	ANOVA
LC	30	25.20-27.20	26.310	0.597	0.109	$p < 0.0001$ Very Highly Significant
LZ	30	21.50-23.90	22.547	0.560	0.102	
CX	30	14.30-16.20	15.370	0.574	0.105	
VP	30	4.80-6.20	5.480	0.433	0.079	
CH	30	0.00-1.90	0.680	0.545	0.099	

[Table/Fig-2]: Comparison between five groups at 24 hours using ANOVA

	n	Range	Mean	SD	SEm	ANOVA
LC	30	26.20-27.90	27.130	0.539	0.098	p < 0.0001 Very Highly Significant
LZ	30	21.70-23.90	22.780	0.592	0.108	
CX	30	15.30-17.20	16.360	0.545	0.099	
VP	29	1.10-2.20	1.569	0.356	0.066	
CH	30	0.00-0.80	0.227	0.264	0.048	

[Table/Fig-3]: Comparison between five groups at 72 hours using ANOVA

Group I (CX) – 2% Chlorhexidine.

Group II (CH) - Calcium hydroxide based intra canal medicament (Ultracal XS®, Ultradent, South Jordan, UT).

Group III (VP) - Vitapex® (Diadent® Group International Inc., Burnaby, B.C.,Canada).

Group IV (LZ) - Linezolid based intra canal medicament (0.3%)

Group V (LC) - Calcium hydroxide and Linezolid based intra canal medicament (3% LZ, 30% CH).

The plates were then incubated overnight at 37°C, after which, the samples were evaluated for zones of inhibition after intervals of 24 hours and 72 hours. The zone of inhibition was measured with a boley guage. The readings corresponding to each medicament were statistically evaluated by paired t-test, ANOVA and Post-hoc analysis using Tukey's HSD.

RESULTS

The difference between values of the zones of inhibition around various medicaments after 24 hours and 72 hours was found to be statistically significant [Table/Fig-1]. The maximum mean value of the zones of inhibition after 24 hours was shown by group LC (26.31 ± 0.597), while the minimum was shown by group CH (0.68 ± 0.545). After 72 hours, the maximum value was again shown by group LC (27.13 ± 0.540), while the least was shown by group CH (0.23 ± 0.264). Groups LC, LZ and CHX showed an increase in their values after 72 hours compared to those after 24 hours. Among these the greatest increase was shown by group CHX, followed by LC and LZ. However, groups VP and CH showed a decline in their values after 72 hours compared to those after 24 hours, out of which the greatest decline was observed for group VP, followed by CH. A comparison between the five groups after 24 hours [Table/Fig-2] (Post-hoc analysis using Tukey's HSD) showed that each group differed significantly from the rest of the groups. A comparison between the five groups after 72 hours [Table/Fig-3] Post-hoc analysis using Tukey's HSD) also showed that each group differed significantly from the rest of the groups.

DISCUSSION

Three different methods have been used to determine the effectiveness of any medicament: Dilution, agar diffusion and direct exposure methods. The dilution method provides quantitative information about the amount of antimicrobial agent required, but has the disadvantage of being able to evaluate only substances that are soluble in the culture media. The direct exposure method provides qualitative information about the substance due to its direct contact with the microorganism being considered. The agar diffusion method presents a zone of inhibition around the wells containing the medicament [12]. It is by far the most commonly used method.

CH releases OH ions that are responsible for the creation of a highly alkaline environment. High pH has a destructive effect on bacterial cell membrane and protein structure [12]. A pH of 10.5 to 11 delays the growth of EF, while, a pH of 11 or more eliminates EF [13,14]. However, CH was the least effective against EF in our study. This is in agreement with a few previous studies [14-16]. The mean zone of inhibition was (0.68 ± 0.545) after 24 hours of incubation, which increased to 0.23 ± 0.264 after 72 hours. When CH is placed in agar, its high pH starts to precipitate it, preventing its diffusion [17]. Moreover, the release of Ca and OH ions decrease the pH of the media, enhancing growth of the organisms being tested [15]. These factors may have been responsible for its lack of effectiveness against EF in blood agar. Moreover, the proton pump of EF carries protons to the interior of the cell, acidifying its cytoplasm in situations of increased alkalinity when subjected to CH [14]. All these factors might have contributed to the pH decline of CH.

Two percent CX has been proven to be more effective than lesser concentrations [18]. That is why it was decided to use this concentration for the study. CX was found to be effective against EF after 24 hours (15.37 ± 0.574) and 72 hours (16.36 ± 0.545) of incubation in agar. This is in accordance with the results obtained by other authors [14,19-21]. However, it is in disagreement with Estrela et al., [22]. It was found to be more effective than CH, which is in agreement with results obtained by Ballal et al., and Gomes et al., [21,23]. However, it was not as effective as LC or LZ.

VP showed effectiveness against EF after 24 hours (5.50 ± 0.426), however, the effect declined after 72 hours (1.57 ± 0.356). The ineffectiveness of VP on organisms has been previously reported [15,24]. In a study by Amorim et al., VP produced no zone of inhibition in the agar diffusion method, however, was found to be effective against EF through direct exposure test [12]. This example confirms the unreliability of the agar diffusion method in assessing VP.

LZ acts by preventing the formation of 70S ribosome complex that is responsible for the initiation of protein synthesis, by binding to the 23S subunit of the 50S subunit [25]. However, enterococcal resistance to the drug occurs due to mutation of the ribosomal binding site [26]. LZ showed good results against EF with the mean zone of inhibition being 22.55 ± 0.560 after 24 hours, and 22.78 ± 0.592 after 72 hours. However, the best results were shown by LC with a mean values of 26.31 ± 0.597 and 27.13 ± 0.540 after 24 hours and 72 hours, respectively. LZ is known to cause adverse effects on systemic administration, like nausea, diarrhea, tongue discoloration, oral moniliasis, taste perversion, headache and myelosupression [27]. However, there has not been an in-vivo study yet evaluating the effectiveness of LZ as an intra canal medicament against EF or any other organism. This in vitro study could not simulate the intra-oral environment inside an infected root canal.

It has been shown that EF rarely appears in primary endodontic lesions [28]. However, some have documented its occurrence in primary as well as secondary endodontic lesions [1,29]. EF can survive inside dentin tubules for atleast 10 days without nutrient supply [16,30]. It may also survive in smear layer and debris, and be extremely difficult to eliminate during instrumentation and irrigation [22]. Moreover, serine protease and ACE aid in the adhesion of EF with dentin [31]. EF has virulence factors (aggregation substance, enterococcal surface proteins (Esp), gelatinase, cytolysin toxin, extracellular superoxide production, capsular polysaccharides, antibiotic resistance determinant) that can facilitate its adherence with host cells and extracellular matrix; help in its invasion into tissues; immunomodulation effect and cause toxin mediated damage [32]. These factors make EF resistant to most intra canal medications.

From this study, we found LC to be promising in eliminating EF in comparison to the other medicaments tested. Hydroxyl ions liberated by CH act on enzymes in the cytoplasmic membrane. The membrane is similar, irrespective of the organisms morphological, tinctorial and respiratory characteristics. This means that CH will have similar effects on aerobic, anaerobic, gram positive and gram negative organisms [12]. Therefore, we recommend the LC combination, because CH could broaden the spectrum of activity beyond the sole gram positive spectrum of action of LZ. But, it might need periodic replacement to ensure absence of any dehydration or reduction in pH of CH, that might invariably affect the potency of LZ.

Endodontic infections are polymicrobial, therefore, a medicament effective against EF need not be as effective in the root canal because the root canal is a habitat for various organisms. Moreover, the agar diffusion method does not distinguish between microbiostatic and microbicidal properties of dental medicaments, neither does it provide information about microbial viability after the test [33]. Also, the test results depend on the medicament's solubility and diffusability in agar, rather than its actual efficacy against the organism. Therefore, we recommend future studies evaluating the effectiveness of the above medicaments using in vivo methods.

CONCLUSION

Within the confines of our study, it was found that LC has the greatest effectiveness against EF, followed by LZ, CX, VP and CH, as evaluated by the agar diffusion method.

REFERENCES

[1] Pirani C, Bertacci A, Cavrini F et al. Recovery of *Enterococcus faecalis* in root canal lumen of patients with primary and secondary endodontic lesions. *New Microbiol*. 2008;31:235-40.
[2] Siqueira JF Jr, Rôças IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2004;97:85–94.
[3] Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. *Int Endod J*. 2001;34:429-34.
[4] Jenkins S, Addy M. The mechanism of action of chlorhexidine. *J Clin Periodontol*. 1988;15:415-24.
[5] Athanassiadis B, Abbot PV, Walsh LJ. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. *Aust Dent J*. 2007;52:64-82.
[6] Menezes MM, Valera MC, Jorge AO, Koga-Ito CY, Camargo CH, Mancini MN. In vitro evaluation of the effectiveness of irrigants and intra canal medicaments on microorganisms within root canals. *Int Endod J*. 2004;37:311-9.
[7] Lana EP, Scelza FZ, Silva LE, Mattos-Guaraldi AL, Junior RH. Antimicrobial activity of calcium hydroxide pastes on *enterococcus faecalis* cultivated in root canal systems. *Braz Dent J*. 2009;20:32-6.
[8] Chai WL, Hamimah H, Cheng SC, Sallam AA, Abdullah M. Susceptability of *enterococcus faecalis* biofilm to antibiotics and calcium hydroxide. *J Oral Sci*. 2007;49:161-6.
[9] Reddy VV, Fernandes. Clinical and radiological evaluation of zinc-oxide eugenol and Maisto's paste as obturating materials in infected primary teeth – Nine months study. *J Indian Soc Pedod Prev Dent*. 1996;14:39-44.
[10] Nurku C, Garcia-Godoy F. Evaluation of a calcium hydroxide iodoform paste (Vitapex®) in root canal therapy for primary teeth. *J Clin Ped Dent*. 1999;23:289-94.
[11] Narang M, Gomber S. Linezolid. *Indian Pediatr*. 2004;41:1129-32.
[12] Amorim FG, Toledo OA, Estrela RA, Decurcio DA, Estrela C. Antimicrobial analysis of different root canal filling pastes used in pediatric dentistry by two experimental methods. *Braz dent J*. 2006;17:317-22.
[13] McHugh CP, Zhang P, Michalek S, Eleazer PD. pH required to kill *Enterococcus faecalis* in vitro. *J Endod*. 2004;30:218-9.
[14] Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. *Int Endod J*. 2002;35:221-8.
[15] Gangwar A. Antimicrobial effectiveness of different preparations of calcium hydroxide. Indian *J Dent Res*. 2011;22:66-70.
[16] Bystrom A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. *Endod Dent Traumatol*. 1985;1:170-5.
[17] Souza-Filho FJ, Soares AJ, Vianna ME, Zaia AA, Ferraz CR, Gomes FA. Antimicrobial effect and pH of chlorhexidine gel and calcium hydroxide alone and associated with other materials. *Braz Dent J*. 2008;19:28-33.
[18] Schafer E, Bossmann K. Antimicrobial effects of chloroxylenol and chlorhexidine in the treatment of infected root canals. *Dtsch Zahnarzti Z*. 2000;55:671-9.
[19] White RR, Hays GL, Janer LR. Residual antimicrobial activity after canal irrigation with chlorhexidine. *J Endod*. 1997;23:229-31.
[20] Heling I, Sommer M, Steinberg D, Friedman M, Sola MN. Microbiological evaluation of the efficacy of chlorhexidine in a sustained release device for dentine sterlization. *Int Endod J*. 1992;25:15-19.
[21] Ballal V, Kundabala M, Acharya S, Ballal M. Antimicrobial action of calcium hydroxide, chlorhexidine and their combination on endodontic pathogens. *Aust Dent J*. 2007;52:118-21.
[22] Estrela C, Estrela RA, Decurcio DA, Hollanda CB, Silva JA. Antimicrobial efficacy of ozonated water, gaseous ozone, sodium hypochlorite and chlorhexidine in infected human root canals. *Int Endod J*. 2007;40:85-93.
[23] Gomes FA, Souza FC, Ferraz CR, et al. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against *Enterococcus faecalis* in bovine root dentin in vitro. *Int Endod J*. 2003;36:267-75.
[24] Blanscet ML, Tordik PA, Goodell GG. An agar diffusion comparison of the antimicrobial effect of calcium hydroxide at five different concentrations with three different vehicles. *J Endod*. 2008;34:1246-8.
[25] Bozdogan B, Esel D, Whitener C, Browne FA, Appelbaum PC. Antimicrobial susceptibility of a vancomycin-resistant *Staphylococcus aureus* strain isolated at the Hershey Medical Center. *J Antimicrob Chemother*. 2003;52:864-68.
[26] Hamel JC, Stapert D, Moerman JK, Ford CW. Linezolid, critical characteristics. *Infection*. 2000;28:60-4.
[27] Ba BB, Arpin C, Nso BB, Dubois V, Saux MC, Quentin C. Activity of linezolid in an in vitro pharmacokinetic-pharmacodynamic model using different dosages and *staphylococcus aureus* and *enterococcus faecalis* strains with and without a hypermutator phenotype. *Antimicrob Agents Chemother*. 2010;54:1443-52.
[28] Sirén EK, Haapasalo MP, Ranta K, Salmi P, Kerosuo NJ. Microbiological findings and clinical treatment procedures in endodontic cases selected for microbiological investigation. *Int Endod J*. 1997;30:91-5.
[29] Sakamoto M, Rocas IN, Siqueira JF, Benno Y. Molecular analysis of bacteria in asymptomatic and symptomatic endodontic infections. *Oral Microbiol Immunol*. 2006;21:112-22.
[30] Siqueira JF Jr. Aetiology of root canal treatment failure: Why well treated teeth can fail. *Int Endod J*. 2001;34:1-10.
[31] Hubble TS, Hatton JF, Nallapareddy SR, Murray BE, Gillespie MJ. Influence of *Enterococcus faecalis* proteases and the collagen-binding protein, Ace, on adhesion to dentin. *Oral Microbiol Immunol*. 2003;18:21-6.
[32] Portenier I, Waltimo T, Haapasalo M. *Enterococcus faecalis*: the root canal survivor and 'star' in post-treatment desease. *Endod Top*. 2003;6:135-9.
[33] Estrela C, Estrela CRA, Bammann LL, Pecora JD. Two methods to evaluate the antimicrobial action of calcium hydroxide paste. *J Endod*. 2001;27:720-3.

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