Original Article

Antimicrobial Efficacy of Medium-Chain Fatty Acids, 2% Chlorhexidine, and 5% Sodium Hypochlorite against *Enterococcus faecalis*: An *in vitro* Study

Krishnapriya Devan, Faizal C. Peedikayil, T. P. Chandru, Soni Kottayi, N. Dhanesh, K. Rahul Suresh[1]

Department of Paedodontics and Preventive Dentistry, Kannur Dental College, Kannur, Kerala, [1]Department of Conservative Dentistry and Endodontics, JKKN Dental College, Komarapalayam, Tamil Nadu, India

Abstract

Background: The current trend globally is to "Go Natural." Medium-chain fatty acids (MCFAs) are natural derivatives with proven antimicrobial properties. *Enterococcus faecalis* is a persistent microbe frequently associated with endodontic treatment failures. Thus, the aim of this study was to compare the antimicrobial efficacy of MCFAs, 2% chlorhexidine, and 5% sodium hypochlorite (NaOCl) against *E. faecalis*. **Materials and Methods:** Lauric acid (LA), decanoic acid (DA), octanoic acid (OA), 2% chlorhexidine, 5% NaOCl, and ethanol were used against pure strains of *E. faecalis*. Six wells of approximately 10 mm were bored in Mueller-Hinton Agar medium using a well cutter and the different test solutions were added. The plates were then incubated at 37°C for 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. **Results:** The results were tabulated and statistically analyzed using analysis of variance and Tukey's *post hoc* tests. There was a statistically significant difference between the six groups compared. Maximum antibacterial activity was shown by 2% chlorhexidine (21.66 mm), followed by LA (17.66 mm) and NaOCl (16.33 mm). The mean zone of inhibition exhibited by DA and OA were 14.00 mm and 12.33 mm, respectively. Least antibacterial activity was shown by ethanol (9.66 mm). **Conclusion:** Within the limitations of the study, it can be concluded that LA exhibited antimicrobial efficacy comparable to that of 5% NaOCl. However, the clinical efficacy of LA must take into account the intricate canal anatomy and polymicrobial nature of root canal infections.

Keywords: *Enterococcus faecalis,* medium-chain fatty acids, root canal irrigant

Introduction

Pulpal disease is one of the prevalent oral diseases of modern times with bacteria or their products entering the pulp being the frequent etiological agents.[1] The root canal flora includes anaerobic bacteria, facultative anaerobic bacteria, and aerobic bacteria. *Enterococcus faecalis*, a facultative anaerobe, is the most commonly implicated microorganism in asymptomatic persistent infections, in root canals exhibiting signs of chronic apical periodontitis and root canal treatment failure cases.[2]

The goal of endodontic therapy is to completely eliminate the microorganisms and their by-products from the root canal system. Chemomechanical debridement of the root canal system can achieve this to an extent, but it is impossible to clean and shape the root canals completely because of the complex anatomy of the root canals. Therefore, irrigation is an inevitable part of the root canal debridement as it allows for cleaning beyond what might be achieved by root canal instrumentation alone.[3]

Access this article online

Quick Response Code:

Website: www.ijohr.org

DOI: 10.4103/ijohr.ijohr_17_18

The most widely used root canal irrigating solution is sodium hypochlorite (NaOCl). It is a potent antimicrobial agent and effectively dissolves pulpal remnants and organic components of dentine when used in concentrations ranging from 0.5% to 5.25%.[1] However, NaOCl has been associated with unpleasant taste, and criticized for its relative toxicity, and inability to remove smear layer.

Chlorhexidine gluconate (CHX), because of its antimicrobial properties, substantivity, and relatively low toxicity, has been in use for a long time in dentistry.[1] Despite these advantages,

Address for correspondence: Dr. Krishnapriya Devan, Department of Paedodontics and Preventive Dentistry, Kannur Dental College, Kannur, Kerala, India.
E-mail: krishnapriyarahul145@gmail.com

How to cite this article: Devan K, Peedikayil FC, Chandru TP, Kottayi S, Dhanesh N, Suresh KR. Antimicrobial efficacy of medium-chain fatty acids, 2% Chlorhexidine, and 5% sodium hypochlorite against *Enterococcus faecalis*: An *in vitro* study. Indian J Oral Health Res 2018;4:47-51.

it has certain drawbacks. Its activity is pH dependent, is greatly reduced in the presence of organic matter, and also lacks the tissue-dissolving ability.

Studies are being conducted regularly in search for an ideal root canal irrigant as the irrigant solutions available to us currently have their share of limitations. During the last few years, there have seen a shift toward deriving newer materials from natural or herbal products owing to their antimicrobial properties with less or no side effects. The medium-chain fatty acids (MCFAs) with aliphatic chains of 6–12 carbons obtained from natural sources are studied extensively for their antimicrobial properties. MCFAs exhibit a broad spectrum of antimicrobial activity.[4] Lauric acid (LA) (C12), decanoic acid (DA) (C10), and octanoic acid (OA) (C8) are the common MCFAs. Rich sources of beneficial MCFAs include coconut oil, palm kernel oil, and butter.[5]

Thus, the aim of this *in vitro* study is to evaluate the antimicrobial efficacy of MCFAs against *E. faecalis* in comparison to 5% NaOCl and 2% chlorhexidine.

Materials and Methods

LA, DA, and OA were purchased from Sigma Aldrich, Bengaluru, India. The MCFAs were compared with 5% NaOCl (Deor, Azure Laboratories Pvt. Ltd., Kochi) and 2% chlorhexidine (Deor, RC chlor, Azure Laboratories Pvt. Ltd., Kochi) as they are the standard irrigating solutions routinely used. About 100% ethanol was used to dissolve the fatty acids and was therefore taken as a control. The test organism *E. faecalis* (ATCC 29212) was obtained from Biogenix Research Centre, Trivandrum, India.

Determination of minimal inhibitory concentration

Minimal inhibitory concentration (MIC) of the three MCFAs against *E. faecalis* was determined using two-fold serial dilution method. The growth of stock inoculum of respective test organisms was adjusted to 1% McFarland Standard. The broth dilution assay was done in 96-well microtiter plate. Each well in the plate were added with 100 µl of the diluted (two times) inoculum suspensions (final volume in each well – 200 µl).

Samples were added in increasing concentrations of 0.25, 0.5, 1, 5, and 10 µg to the respective wells and incubated overnight at room temperature. A control well was kept with organism alone.

Growth was observed by visual inspection and by measuring the optical density (OD) at 630 nm using ELISA plate reader. The OD was measured immediately after the visual reading. The growth inhibition for the test wells at each extract dilution was determined by the formula:

Percentage of inhibition = ([OD of control − OD of test]/[OD of control]) ×100%.

After the MIC values were read, the inhibitory concentration at 50% (IC_{50}) of the microbial strains was calculated. The MIC of LA, DA, and OA were determined to be 2.6 µg/ml, 3.3 µg/ml, and 1 µg/ml, respectively [Table 1].

Preparation of the agar medium

The agar medium was prepared by dissolving 33.8 g of the commercially available Muller-Hinton Agar medium (MHI Agar media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 min. The autoclaved medium was mixed well and poured onto 100-mm Petri plates (25–30 ml/plate) while still molten.

Determination of the antimicrobial activity

The antimicrobial activity of MCFAs against *E. faecalis* was determined by agar well-diffusion method. Petri plates containing 20-ml Muller-Hinton Agar medium were seeded with bacterial culture of *E. faecalis* (growth of culture adjusted according to the McFarland Standard, 0.5%). Six wells of approximately 10 mm were bored using a well cutter and the different test solutions were added. Three such inoculation plates were prepared. The plates were then incubated at 37°C for 24 h. The antimicrobials present in the samples were allowed to diffuse out into the medium and interact with the test organism. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells [Figure 1].

Statistical analysis

The data obtained were subjected to statistical analysis (Statistical Package for the Social Sciences for Windows, SPSS 17, IBM Corporation, Chicago, US). Analysis of variance (ANOVA) was used to compare the six groups and to compare the groups in pairs, Tukey's *post hoc* test was done.

Results

ANOVA showed that there was a statistically significant difference between the six groups compared [Table 2]. Maximum antibacterial activity was shown by 2% chlorhexidine (21.66 mm) followed by LA (17.66 mm) and NaOCl (16.33 mm). Tukey's *post hoc* test [Table 3] showed that there was statistically significant difference in the inhibition zone diameter of the groups compared except for that of LA and 5% NaOCl and DA (14.00 mm) and OA (12.33 mm). Least

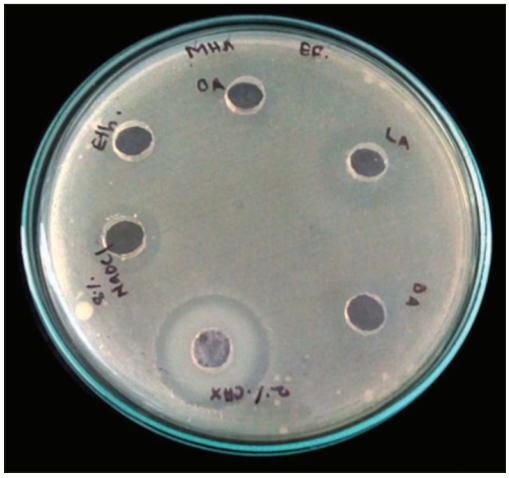

Figure 1: Zone of inhibition exhibited by different agents

Table 1: Minimal inhibitory concentration values of lauric acid, decanoic acid, and octanoic acid against *Enterococcus faecalis*

	Enterococcus faecalis					
Concentration (μg)	**OD630**			**Percentage inhibition**		
	LA	**DA**	**OA**	**LA**	**DA**	**OA**
Control	0.306	0.306	0.306	0	0	0
0.25	0.1706	0.1993	0.1543	44.61028192	35.09121	50.01658
0.5	0.1549	0.1795	0.1439	49.81757877	41.65837	53.466
1	0.1541	0.1586	0.1323	50.08291874	48.59038	57.31343
5	0.1459	0.1184	0.132	52.8026534	61.92371	57.41294
10	0.136	0.113	0.1068	56.08623549	63.71476	65.77114
MIC value				2.6 μg	3.3 μg	1 μg

MIC: Minimal inhibitory concentration, OA: Octanoic acid, LA: Lauric acid, DA: Decanoic acid

Table 2: Readings of mean zones of inhibition and analysis of variance

Agent	*n*	Mean (mm)	Mean±SD	ANOVA
Ethanol	3	9.6667	9.6667±0.57735	*P*=0.000 <0.05 (significant)
LA	3	17.6667	17.6667±0.57735	
DA	3	14.0000	14.0000±0.1.000	
OA	3	12.3333	12.3333±1.1547	
2% chlorhexidine	3	21.6667	21.6667±0.57735	
5% sodium hypochlorite	3	16.3333	16.3333±0.57735	

n: Number of plates, SD: Standard deviation, ANOVA: Analysis of variance, OA: Octanoic acid, LA: Lauric acid, DA: Decanoic acid

antibacterial activity was shown by ethanol with a mean zone of inhibition diameter of 9.66 mm.

Discussion

E. faecalis was selected for the present study because it is a microbe resistant to elimination by disinfecting agents and a common causative agent for reinfection. *E. faecalis* can survive harsh environments such as extreme alkaline pH, salt concentrations, temperature of 60°C, and resists degradation by bile salts, detergents, heavy metals, ethanol, azide, and desiccation.[6] *E. faecalis* has a prevalence of 40% in primary endodontic infection and 24%–77% in persistent endodontic infection.[7,8]

NaOCl is an effective irrigant against *E. faecalis* including its existence as biofilm. Another irrigant that is commonly used is CHX. However, both these irrigants have their limitations.

There has recently been a renewed interest in the antimicrobial effects of natural compounds used as health remedies until the advent of antibiotic drugs in the 1940s and 1950s.[9] With the emergence of drug-resistant bacterial and viral strains, the antimicrobial actions of natural compounds have been studied by modern scientific methods.

MCFAs are derived from natural sources. They are considered to be environmentally safe and generally harmless to the body in concentrations which kill pathogenic microbes.[9] The antimicrobial effects of MCFAs against bacteria, fungi, viruses, and protozoa have been investigated extensively.

The two possible molecular mechanisms[9] to account for the antimicrobial action of fatty acids are as follows:

1. Alteration of the biochemical functions and loss of viability by a specific interaction with sites within the microorganism or
2. Disturbance in the structure of the microorganism by general nonspecific interaction and thereby inhibiting normal physiological function.

The minimum inhibitory concentration of the materials to be tested was done using the broth microdilution method. Broth microdilution test was chosen for determination of MIC in this study because it is reproducible, easy to perform as channels are prepared, cost-effective, and saves reagents and space.

There are different approaches to test the effectiveness of antimicrobial agents proposed by different authors. Ohara *et al*.[10] and D'Arcangelo *et al*.[11] used growth of selected bacteria as lawns on agar surfaces. The disc diffusion method was used by Siqueira *et al*.,[12] Briseno *et al*.,[13] and Sen *et al*.[14] used the artificial infection of extracted teeth with selected bacteria and *in situ* irrigation with the test antimicrobial agents. In the present study, we have used the agar well-diffusion method as a preliminary *in vitro* assessment to determine whether further investigation is warranted. Under routine laboratory conditions, agar diffusion method is the generally accepted procedure for determining *in vitro* sensitivity.[15] The advantages of agar diffusion method are that it is simple to perform, relatively reproducible, direct, well-controlled and allows bacteria to grow in a simple biofilm on the agar surface and the results can be obtained in a short period of time.

In the present study, all the MCFAs showed inhibition of *E. faecalis* growth. The inhibitory action of fatty acids may be due to their surfactant activity and their ability to cause cellular lysis by disrupting cell membranes.[16] Of the three MCFAs, LA showed the highest inhibitory activity. Studies by Hess *et al*.[17] and Hinton *et al*.[18] reported inhibitory action of LA

Table 3: Tukey's *post hoc* test

		Tukey HSD		
I	J	Mean difference (I − J)	SE	Significant
Ethanol	LA	−8.00000*	0.63828	0.000
	DA	−4.33333*	0.63828	0.000
	OA	−2.66667*	0.63828	0.013
	2% chlorhexidine	−12.00000*	0.63828	0.000
	5% NaOCl	−6.66667*	0.63828	0.000
LA	Ethanol	8.00000*	0.63828	0.000
	DA	3.66667*	0.63828	0.001
	OA	5.33333*	0.63828	0.000
	2% chlorhexidine	−4.00000*	0.63828	0.000
	5% NaOCl	1.33333	0.63828	0.353
DA	Ethanol	4.33333*	0.63828	0.000
	LA	−3.66667*	0.63828	0.001
	OA	1.66667	0.63828	0.168
	2% chlorhexidine	−7.66667*	0.63828	0.000
	5% NaOCl	−2.33333*	0.63828	0.030
OA	Ethanol	2.66667*	0.63828	0.013
	LA	−5.33333*	0.63828	0.000
	DA	−1.66667	0.63828	0.168
	2% chlorhexidine	−9.33333*	0.63828	0.000
	5% NaOCl	−4.00000*	0.63828	0.000
2% chlorhexidine	Ethanol	12.00000*	0.63828	0.000
	LA	4.00000*	0.63828	0.000
	DA	7.66667*	0.63828	0.000
	OA	9.33333*	0.63828	0.000
	5% NaOCl	5.33333*	0.63828	0.000
5% NaOCl	Ethanol	6.66667*	0.63828	0.000
	LA	−1.33333	0.63828	0.353
	DA	2.33333*	0.63828	0.030
	OA	4.00000*	0.63828	0.000
	2% chlorhexidine	−5.33333*	0.63828	0.000

*The mean difference is significant at the 0.05 level. HSD: Honestly significant difference, SE: Standard error, NaOCl: Sodium hypochlorite, OA: Octanoic acid, LA: Lauric acid, DA: Decanoic acid

on *E. faecalis* biofilm formation. According to Ja-Hyung and Young-Wook,[19] high antimicrobial activity of LA could be due to the inhibition of microbial survival and biofilm growth. Padgett[20] *et al*. reported that high level of LA addition (8%) significantly lower the biofilm water permeability. Compared to DA and OA, LA exhibited better antimicrobial activity may be because of the difference in the carbon chain length. Even though DA exhibited better antibacterial effect against *E. faecalis* when compared to OA, the result was not statistically significant. Literature search revealed very few studies comparing the antimicrobial efficacy of all the three MCFAs on *E. faecalis*.

Comparing the six groups, the maximum antibacterial activity was shown by 2% chlorhexidine followed by LA and 5% NaOCl. The difference in the mean zone of inhibition diameter between LA and 5% NaOCl was not found to be statistically significant.

In the present study, the fatty acid solutions were prepared in ethanol stock solutions similar to the procedure followed by Batovska *et al*.[21] and Huang *et al*.[22] in their studies. Ethanol also showed some amount of inhibitory activity in the present study. Thus, the inhibitory activity shown by the MCFAs could be a synergistic action with ethanol. This is similar to the findings of Huang *et al*.[22] that the bactericidal or bacteriostatic activity of the fatty acids could be enhanced in the presence of ethanol. However, this does not undermine the importance of the observations of the effect of these fatty acid solutions.

Agar diffusion method cannot be used to determine the efficacy of a process *in vivo* because in the mouth, bacteria grow in complex biofilms.[15,23] The biofilm itself has different physical and chemical properties. Hence, the use of an oral biofilm model might be considered a more appropriate means of simulating the oral environment for assessing antimicrobial agents. Future studies should also be directed in checking properties other than the antimicrobial efficacy such as tissue dissolution and biocompatibility of these MCFAs before they can be introduced as irrigants clinically.

Conclusion

Within the limitations of the study, it can be concluded that LA exhibited significant antimicrobial activity against *E. faecalis*. The action of LA was comparable to that of 5% NaOCl. Maximum antibacterial activity was shown by 2% chlorhexidine. The action of DA and OA was significantly less than that of LA. Even though the *in vitro* observation of antimicrobial activity of LA appears to be promising, further preclinical and clinical trials have to be done to check the biocompatibility and safety, for it to be used as an intracanal irrigant.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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