Volume : 4 | Issue : 4 | April 2015 • ISSN No 2277 - 8179

Research Paper

A Comparative Evaluation of the Antimicrobial Efficacy of Three Different Intracanal Irrigants Against Enterococcus Fecalis – An in Vitro Study.

Medical Science

KEYWORDS : Chlorhexidine Digluconate, Enterococcus fecalis, Minimum Inhibitory Concentration, Sodium Hypochlorite.

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ABSTRACT

Enterococcus fecalis is a gram positive facultative anaerobe that is rarely seen to be present in primary endodontic infections, but is the most common cause of secondary endodontic infections.

The following study compares the antimicrobial efficacy amongst 2% Tea Tree Oil, 2% Chlorhexidine Digluconate, 3% Sodium Hypochlorite and the control (Distilled Water) using the Minimum Inhibitory Concentration(MIC) Test. The MIC was performed using 10-fold dilution in 96 U-Well Micro Test plates.

The results were tabulated and statistically analyzed using binary statistics. It was seen that Tea Tree Oil was the most effective in inhibiting E. fecalis, followed by Sodium Hypochlorite, and Chlorhexidine Digluconate was the least successful. Distilled water showed no effect on the gram positive organisms.

INTRODUCTION

Root canal treatment is performed to eliminate the organisms and prevent their recolonization within the tooth. Even after proper endodontic treatment, bacteria such as Enterococcus fecalis can survive and grow[1].

It is able to form biofilms and invade dentinal tubules[2]. This microorganism is also known to be the species commonly recovered from the root canal treated teeth.[3,4] Furthermore, only 33% of the teeth, which harbor Enterococcus fecalis when the canals are being refilled, have demonstrated endodontic success[4]. Evans *et al* showed that it is resistant to Calcium Hydroxide because of its property of tolerance to alkaline conditions[5]. Therefore the presence of Enterococcus fecalis at the time of canal filling lowers the rate of treatment success to a great extent[6].

The anatomy of the root canal system, and invasion of the dentinal tubules by microorganisms, are the major obstacles in achieving the primary objectives of complete cleaning and shaping of root canal systems.

Proper choice of antimicrobial irrigants is an important step to decrease the number of microorganisms, which has to be considered. Mechanical effects of irrigants are generated by the back and forth flow of the irrigation solution during cleaning and shaping of the infected root canals significantly reducing the bacterial load[7].

To effectively clean and disinfect the root canal system, an irrigant should be able to disinfect and penetrate dentinal tubules, offer long-term antibacterial effect, remove the smear layer, and should be non antigenic, nontoxic and noncarcinogenic. Other desirable properties for an ideal irrigant include the ability to dissolve pulp tissue and inactivate endotoxins[8].

Chlorhexidine gluconate is used at a concentration of 2% to 6% as a root canal irrigant. It has a broad spectrum anti-bacterial action, substantively low toxicity, lack of foul smell and bad taste[9,10]. Due to these properties, it has been recommended as a potent root canal irrigant[10,11]. Chlorhexidine has shown to be more effective against gram positive organisms than gram negative organisms[11].

Sodium hypochlorite is the most commonly used root canal irrigant. It has been used in dilutions ranging from 0.5% to 5.25%. Free chlorine in Sodium Hypochlorite (NaOCl) dissolved vital and necrotic tissue by breaking down proteins into amino acid, decreasing the concentration of the solution, reduces its toxicity, anti-bacterial effect and ability to dissolve tissues.

The major disadvantages of this irrigant are its cytotoxicity when accidentally introduced into the periradicular tissues, foul smell, taste, and corrosion of metal objects.

With the advent of time, researchers have constantly looked for newer root canal irrigants, which have an improved antimicrobial action, and are non-toxic to the oral tissues. Tea tree oil has a broad-spectrum antimicrobial, antifungal, antiviral, antioxidant and anti-inflammatory effect. Tea tree oil has been in use as an anti-microbial in the field of dermatology and internal healing for centuries.

Contemporary endodontic literature lacks sufficient evidence and clinical research on Tea Tree Oil as an endodontic irrigant, hence, the present study was designed to compare its efficiency against an established endodontic microorganism, E. fecalis,

Volume : 4 | Issue : 4 | April 2015 • ISSN No 2277 - 8179

along with routine endodontic irrigants.

METHODOLOGY

The study aimed to compare the antimicrobial efficacy of 2% Chlorhexidine Digluconate(Dentochlor, India), 3% stabilized solution of Sodium Hypochlorite (Parcan, Septodont, France) and a 2% prepared solution of Tea Tree Oil (The Tea Tree Therapy, USA) on E. Fecalis.

Two parts of 100% pure Tea Tree Oil were mixed with 98 parts of distilled water to obtain 2% concentration of Tea Tree Oil. The stock solutions of chlorhexidine digluconate and sodium hypochlorite was transferred to the 10mL bottles without any alteration .Distilled water was used as control. A suspension was prepared by mixing a pure culture of E fecalis ATCC 29212, grown in nutrient agar plates for 24h, with 2mL of sterile 0.85% saline solution. The suspension was adjusted to achieve turbidity equivalent to 0.5 McFarland turbidity standards. A ten-fold dilution was carried out of the antibiotic solution with the broth containing the E fecalis, which has been dispensed in the U-wells.The Minimum Inhibitory Concentration (MIC) was observed to clearly differentiate the extent to which the reagents were effective in eliminating E fecalis.

The inoculation of the samples were done in the following manner, and the trays were labelled accordingly-

Distribution of samples

Group I – Untreated Control Group - Distilled water

Group II – 2% Chlorhexidine Digluconate

Group III – 3% Sodium Hypochlorite

Group IV – 2% Tea tree Oil

The microdilution trays were incubated at 37^0C for 48 hours in an ambient air incubator. After 48 hours, the trays were taken out and adequate light was used to visually compare the wells. Each well was individually compared with the negative control and the point where the turbidity is reduced to be such that it is comparable to the negative control, was noted.

This is the point where the complete inhibition of the microorganism occurred, as it is the point at which the concentration of the reagent is adequate to completely inhibit the microbial growth. Since the CFU's/ml were constant in all the wells, it was the concentration of the reagent of this particular well that was noted and taken into account, to further determine the minimum reagent required to adequately inhibit any microbial activity.

RESULTS

In the wells in which Tea Tree Oil was microdiluted, it was seen that no growth of E. fecalis was found till the dilution was diminished to a concentration of 2×10^{-12} ml. Sodium Hypochlorite was seen to be effective till a concentration of 3×10^{-10} ml and Chlorhexidine Digluconate was effective until a concentration of 2×10^{-7}ml. Distilled water was unable to inhibit the growth of E fecalis in any of the wells where it was microdiluted.

Table I: Testing of significance of Irrigants considered in our study for their Efficacy on E Faecalis using Chi- Square Test

		Effect		Total	Chi-square (χ^2) value	p-value
		No Colonies Visible	Colonies Visible			
Irrigants	Distilled Water	0	100	100	154.476	< 0.0001
	Tea Tree Oil (2%)	80	20	100		
	Sodium Hypochlorite Solution (3%)	60	40	100		
	Chlorhexidine Digluconate (2%)	27	73	100		
Total		167	233	400		

We can see(Table1) that the value of Chi square statistic is very high, hence p-value is very small. As p-value is less than 0.05, we have strong evidence that there is a significant effect of the irrigants under observation on E Faecalis.

DISCUSSION

Minimum inhibitory concentrations (MIC's) are defined as the lowest concentration of an anti-microbial that will inhibit the visible growth of a microorganism after overnight incubation. MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool used to determine MIC breakpoints.

In the present study, 2% Chlorhexidine digluconate and 3% Sodium Hypochlorite were used, as they are commonly used irrigants during endodontic treatment, especially for the removal of E. fecalis. Tea tree oil (Group IV) is the new product that has previously seen to diminish bacterial colonies when used topically on skin and nails, but its effect in the root canal is yet to be evaluated.

Similar conditions have been tried to be replicated in this study by having sterilized 96 U-well micro test plate containing only Enterococcus fecalis ATCC 29212 strain as the bacterium. The ability of tea tree oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the most likely source of its lethal action at minimum inhibitory levels.

Tea tree oil showed a statistically significant reduction in the bacterial colonies, which was seen to be better than the performance of chlorhexidine and even sodium hypochlorite. The antimicrobial action of Tea tree oil results from its ability to disrupt the permeability barrier of microbial membrane structures.

Chlorhexidine digluconate is a cationic bisguanide that seems to act by adsorption onto the cell wall of the microorganism and causing leakage of intracellular components

Sodium Hypochlorite is widely used in endodontics as a root canal irrigant at different concentrations, which range from 0.5% to 6%. Laboratorial and clinical investigations have shown that it produces an effective chemomechanical debridement of the root canal system, due to its properties, such as lubricating action for instrumentation, antimicrobial activity and dissolution of pulp tissue. The usage of NaOCl in high concentrations is undesirable because it is irritating to the periapical tissues.

Therefore, several attempts have been made in order to find other efficient irrigants that provide a high antimicrobial action with low toxicity.

Even though Tea tree oil is found to be the most effective irrigant in this study, it would only be right to consider it for use in clinical practice once it is adequately tested for its other properties as well, other than its antimicrobial property, which are, its toxicity and biocompatibility, for which further studies are recommended.

CONCLUSION

The present study made an attempt to compare the effectiveness of three intracanal irrigants, namely, Chlorhexidine Digluconate, Sodium Hypochlorite, and Tea tree Oil, in the elimination of E. fecalis from the root canal. Within the limitations of the current study, the following conclusions were drawn based on the findings:

1. Distilled Water showed no effect in the elimination of E. fecalis.
2. Tea tree Oil was found to be the most effective irrigant in the elimination of E. fecalis, followed by Sodium Hypochlorite. Chlorhexidine Digluconate, was the least effective irrigant.

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