IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 7 Ver. 14 (July. 2018), PP 39-42
www.iosrjournals.org

Comparative Evaluation of the Antimicrobial Efficacy of Chlorhexidine, Octenidine and Sodium Hypochlorite against E. Fecalis: An In-Vitro Study.

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Abstract:*Enterococcus fecalis is a gram positive facultative anaerobe which is rarely present in primary endodontic infections, but is the most common cause of secondary endodontic infections. The following study compares the antimicrobial efficacy of 0.2% Octenidine, 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 0.2% Octenidine 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.*
Key words: *root canal disinfection, antimicrobial property, chlorhexidine, Octenidine, sodium hypochlorite.*

Date of Submission: 10-07-2018 Date of acceptance: 27-07-2018

I. Introduction

The primary objective of root canal treatment is to eliminate the micro organisms and prevent their recolonization within the tooth. However, after thorough cleaning and shaping of the root canals Enterococcus fecalis can thrive inside the tooth[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 root 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 hindrances in achieving the primary objectives of complete cleaning and shaping of root canal systems thus making use of a potent antimicrobial irrigant imperative to its success. Irrigants also act by mechanical effects which are generated by the back and forth flow of the irrigation solution during cleaning and shaping thereby 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. 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. Octenidine hydrochloride (OCT) Octenidine (Schulke and Mayr GmbH, Norderstedt, Germany), is a bispyridine derivative, that is, N, N- [1,10-decanediyldi- 1 (4H)-pyridinyl-4pylidene] bis (1- octanamine) dihydrochloride. The existing data suggest that a mouthrinse containing 0.1% OCT may be capable of exerting beneficial clinical effects upon plaque accumulation and gingivitis. OCT used in the form of mouthrinse was reported to inhibit dental plaque and caries both in rats[12] and humans. It has been demonstrated that OCT appears to be more effective than chlorhexidine as a means for prolonged bacterial anti-adhesive activity. [13,14] OCT has been suggested as an endodontic irrigant based on its antimicrobial effects and lower cytotoxicity.

II. 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 0.2% prepared solution of Octenidine on E. Fecalis. pure Octenidine was mixed with absolute alcohol to obtain 0.2% concentration of Octinidine. The stock solutions of chlorhexidine digluconate and sodium hypochlorite were 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 was 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 – 0.2% Octinidine.

The microdilution trays were incubated at 370 C 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 this 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.

III. Results

In the wells in which 0.2% Octenidine was microdiluted, it was seen that no growth of E. fecalis was found till the dilution was diminished to a concentration of 0.2×10^{-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 (c2) value	P-value
		No Colonies Visible	Colonies Visible			
Irrigation Regime	Distilled Water	0	100	100	154.476	< 0.0001
	Octenidine (0.2%)	83	17	100		
	Sodium hypochlorite Solution (3%)	52	48	100		

	Chlorhexidine Digluconate (2%)	**27**	**73**	**100**		
Total		**162**	**238**	**400**		

As shown in Table I 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.

IV. 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. 0.2% Octenidine (Group IV) is the new productwhich is similar to Chlorhexidine, but its effect in the root canal is yet to be evaluated. Similar conditions have been replicated in this study by having sterilized 96 U-well micro test plate containing only Enterococcus fecalis ATCC 29212 strain. OCT has been demonstrated to be more effective than chlorhexidine as a means for prolonged bacterial antiadhesive activity. Octenidine may still be useful as an alternative endodontic irrigant. Its excellent antimicrobial properties support this inference. Octenidine 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 Octenidine 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 Octenidine 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.

V. Conclusion

The present study compared the effectiveness of three intracanal irrigants, namely, Chlorhexidine Digluconate, Sodium Hypochlorite, and Octenidine , 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. Octenidine 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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Dr. Akriti Goel "Rative Evaluation Of The Antimicrobial Efficacy Of Chlorhexidine, Octenidine And Sodium Hypochlorite Against E. Fecalis: An In-Vitro Study. "IOSR Journal Of Dental And Medical Sciences (IOSR-JDMS), Vol. 17, No. 7, 2018, Pp 39-42.