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Antimicrobial Efficacy of Chlorhexidine and MTAD against Enterococcus Faecalis - An *In Vitro* Study

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Abstract **Original Research Article**

Aim of the study: To compare the antimicrobial efficacy of Chlorhexidine and MTAD against Enterococcus faecalis in dentinal tubules of human teeth**.** ***Materials and Method:*** Freshly extracted, single rooted tooth were immersed in 5.25% NaOCl to remove surface soft tissue and organic debris. The teeth were horizontally sectioned into coronal, middle and apical sections. The 5 mm middle segment was used for the study. The segments were placed in brain-heart infusion broth containing a culture of E.faecalis. The root specimens were randomly divided into 3 groups. To test for bacterial survival, dentinal shavings from within the canal were collected using round bur on a piece of sterile aluminium foil. The mean CFU/mg and standard deviation value were calculated. ***Results*****:** The antibacterial property of MTAD against E. faecalis was superior compared to that of Chlorhexidine.

Keywords: antimicrobial efficacy, Chlorhexidine, MTAD, E.faecalis.

INTRODUCTION

Microorganisms play a fundamental role in the etiology of pulp and periapical diseases, their control and elimination are important during endodontic treatment [1]. Disinfection of the root canal is the major determinant in the healing of periapical tissues [2]. Clinical studies have demonstrated that chemo-mechanical preparation and use of antimicrobial medicaments are effective in reducing the bacterial load in root canal systems [3]. However some bacteria can still persist despite these efforts. These bacteria are primarily facultative anaerobic gram-positive species, mainly Enterococcus faecalis [4].

E. faecalis is persistently found in root canal failures is able to survive in the root canal as a single organism or as a major component of the flora and is resistant to various intracanal medicaments [5].

Chlorhexidine (CHX) a broad spectrum antimicrobial is widely used as a mouth rinse in the prevention and treatment of periodontal diseases and dental caries, it has been suggested as an irrigating solution or intracanal dressing in endodontic therapy[6]. CHX exhibits a property of substantivity and has low grade of toxicity. But it is unable to dissolve necrotic tissue remnants [7]. Because of these limitations, a search for better root canal irrigant is continued.

A new irrigating solution, MTAD containing a mixture of a tetracycline isomer, an acid, and a detergent has shown promising results. Recent investigations have demonstrated its ability to safely remove the smear layer and effectively eliminate E. faecalis [8, 9].

The aim of the present in vitro study was to compare the antimicrobial efficacy of 2% Chlorhexidine and MTAD against Enterococcus faecalis.

MATERIALS AND METHODS

METHODOLOGY

Sample Size

A total of seventy samples were taken for the study. They were divided into two groups of thirty in each and a control group of ten samples.

Method of Study

CRITERIA FOR SELECTION OF SAMPLES

Inclusion Criteria

1. Tooth should be non- carious
2. Tooth should be intact with straight root

Exclusion Criteria

1. Carious tooth
2. Root canal treated tooth

Freshly extracted, single rooted tooth were immersed in 5.25% NaOCl to remove surface soft tissue and organic debris. The teeth were horizontally sectioned into coronal, middle and apical sections using a carbide disc in a straight hand-piece. The 5 mm middle segment was used for the study. The root canal of each specimen was enlarged with a # 10 round bur to standardise the internal diameter of the canal. The smear layer, including organic and inorganic debris, was removed using 17% EDTA solution followed by rinsing with 5.25% NaOCl each for 5 minutes. The segments were then sterilised by autoclaving at 121^0 C for 30minutes. The segments were placed in brain-heart infusion broth containing a culture of E.faecalis and incubated for 5 days at 35^0 C to infect the dentinal tubules. After 5 days, the segments were removed from the broth, rinsed with sterile water and blotted dry with sterile gauze. The root specimens were randomly divided into groups and glued upright in petri dishes using a quick setting epoxy resin.

In Group 1 -30 samples 2% chlorhexidine was placed in the canal space of the segment

Group 2-30 samples BioPure MTAD was placed in the canal space of the segment

Group 3- 10 samples kept as a control in which saline was placed.

The groups were kept for incubation at 35^o C and 100% humidity for 1 week. At the end of 1week, the segments were removed from the petri dishes and irrigated with 2ml of sterile water and dried with gauze and paper points. To test for bacterial survival, dentin shavings from within the canal were collected using round burs of increasing diameter and collected on a piece of sterile aluminium foil and weighed. The dentinal shavings were suspended in a solution. 100 of this was pippetted out and poured onto Mc Conkey's agar. This was streaked using a sterile metal loop to spread the suspension evenly throughout the agar plate. They were incubated for 24 hours and colony forming units (CFU) were enumerated .Using the recorded weight of dentin shavings, the number of CFU/mg of dentin was determined. The mean CFU/mg and standard deviation values were calculated for Groups.

STATISTICAL ANALYSIS

Data were presented in terms of mean and SD. Comparison of antimicrobial efficacy of all groups was done by Analysis of variance (ANOVA) followed by post hoc test Dunnett's t-test compared with control group. A p-value less than 0.05 were considered as significant. Data analysis was done by software Minitab v14.0.

RESULTS

Table-1: Basic characteristics of groups

	N	Min	Max	Mean	SD	SE
Group 1	30	72×10^3	114×10^3	92.8×10^3	10.23×10^3	1.86×10^3
Group 2	30	36×10^3	82×10^3	49.3×10^3	11.65×10^3	2.13×10^3
Group 3	10	162×10^3	182×10^3	172.2×10^3	7.63×10^3	2.41×10^3

Table-2: Comparison of antimicrobial efficacy of CHX and MTAD against Saline in dentinal tubules of human teeth (mean±SD)

	Group 1	Group 2	Group 3	F-value	p-value
CFU/mg (10^3)	92.8 ± 10.2 **	49.3 ± 11.6 **	172.2 ± 7.6	439.1	P<0.0001

*p<0.05, **p<0.01, compared with control, Dunnett's t-test is used after analysis of variance (ANOVA).Comparison of all groups shows statistically highly significant (p<0.0001). Post hoc analysis reveals that all experimental groups shows significant difference from control group (p<0.01). Based on result, group 2 showed better antimicrobial efficacy among groups.

DISCUSSION

A number of factors may present obstacles in achieving complete disinfection of the root canal system. The root canal morphology in human teeth is complex and contains many fins, cul-de-sacs and lateral canals. Bacteria may be present not only in these irregularities but also in the dentinal tubules at varying depths [10].

E. faecalis is capable of penetrating as far as 250 µm into the dentinal tubules thus enabling it to resist the action of irrigants. The organism E.faecalis, a Gram-positive facultative anaerobe was selected in this study because it is commonly found in the root canals of failing endodontically treated cases and is resistant to currently used chemicals such as sodium hypochlorite, potassium iodide or calcium hydroxide and has been found to survive as a monoinfection in the root canals [11, 12].

Chlorhexidine is a cationic bis-biguanide with broad spectrum antibiotic activity, and has been widely used in dentistry. CHX readily dissociates at physiological pH, releases positively charged molecules that bind to negatively charged phosphate groups on the microbial cell walls of the bacteria and alters the cell's osmotic equilibrium. CHX has unique ability to adsorb onto the dentin and prevent microbial colonisation (substantivity) over a period of time [13].

MTAD (mixture of tetracycline isomer, an acid and a detergent), after its introduction in 2003 was

subjected to various test procedures to evaluate its efficacy and was compared with various commonly used irrigants. The superior antimicrobial activity of MTAD over 3% NaOCl seen in this study are in agreement with the findings of Shabahang and Torabinejad's study [11].

In present study the smear layer was removed before contaminating the teeth with E.faecalis to allow penetration of bacteria into the tubules. Previous in vitro studies have shown a high level of susceptibility of E.faecalis to MTAD, even when this solution was diluted 200 times, whereas NaOCl loses its antibacterial activity against the same isolate beyond 32 times dilution [9]. MTAD removes the smear layer with significantly less erosion of the dentinal tubules compared to EDTA [8]. Also when MTAD was evaluated for biocompatibility, it was found to be less cytotoxic than Eugenol, 3% H_2O_2, Ca $(OH)_2$ paste, 5.25% NaOCl, 0.12% Chlorhexidine gluconate, and EDTA and more cytotoxic than 2.63%, 1.31% and 0.66% NaOCl [14]. The antimicrobial efficacy of MTAD is because of anticollagenase activity of Doxycycline, its low pH, and its ability to be released gradually over time[8]. Its action is also facilitated by citric acid which removes the organic and inorganic substances. Tween-80 the detergent present in MTAD reduces the surface tension on the dentinal tubules and allows deeper penetration of Doxycycline into the tubules.

One of the significant features of MTAD is its capacity to kill E.faecalis after a mere exposure of 5 min making it highly advntages in the clinical situation. However this effect was not seen with NaOCl [9]. Newberry *et al*., showed that MTAD inhibited most strains of E.faecalis growth when diluted 8192 times and killed most strains of E.faecalis at dilution of 512 times [15]. Thus MTAD has superior antibacterial activity.

The outcome of the study was merely to differentiate the antimicrobial activity of various irrigating solutions against Enterococcus faecalies. MTAD found to be superior against E. faecalies within the limitation of this study, which also requires to be adjudged in combination with other irrigating solutions.

Laboratory tests of any kind are only the first steps in a study of the effectiveness of irrigants. Antibacterial activity of an in vitro environment depends upon the p^H of the substrates in plates or tubes, sensitivity of the drug, bacterial source (wild strains or collection species), the number of bacteria inoculated, incubation time, and the metabolic activity of the microorganisms. The clinical implications of these results could be better understood if additional studies are performed using biofilms and other microorganisms found in the infected root canals.

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