Comparative efficacy of antimicrobial agents used in endodontic treatment on selected microorganisms.

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Abstract: This study was undertaken to compare and evaluate the antimicrobial efficacy of Biopure MTAD a root canal irrigant used for final rinse and commonly used intracanal medicament Metapex against Enterococcus faecalis and Propionibacterium acne. Agar disc diffusion test was done to compare and evaluate antimicrobial efficacy of Biopure MTAD (Dentsply Tulsa Dental, Tulsa, OK) and Metapex (META BIOMED CO.LTD, Korea). Pre-sterilized Whatman paper discs, 6 mm in diameter and soaked with the test solution, were prepared and placed onto the previously seeded agar Petri plates. Each plate was incubated at 37°C for 48 hours. A zone of inhibition was recorded for each plate and the results were analysed statistically. Saline and Ethanol used as control group in this study. Biopure MTAD was more effective than Metapex against Enterococcus faecalis and Propionibacterium acne with a substantial zone of inhibition. Statistically significant (p<0.5) difference were seen in zone of inhibition size between tested antimicrobial agents against E.faecalis but nonsignificant (p>0.5) difference in zone of inhibition size against P.acne was found between Biopure MTAD and Metapex. Biopure MTAD was found to be superior in its antimicrobial abilities compared to Metapex.

Keywords: agar disc diffusion test, biopure MTAD, E.faecalis, metapex.

Introduction

Microorganisms and their by-products are considered to be the major cause of pulp and periradicular pathosis. Therefore the major objective in root canal treatment is to disinfect the entire root canal system, which requires that all contents of the root canal system be eliminated as possible sources of infection. This goal may be accomplished by using mechanical instrumentation and chemical irrigation, in conjunction with medication of the root canal system between treatment sessions (1,2).

Several studies using advanced techniques such as microcomputed tomography (CT) scanning have demonstrated that proportionally large areas of the main root-canal wall remain untouched by the instruments (3), emphasizing the importance of chemical means of cleaning and disinfecting all areas of the root canal.

During and after instrumentation, the irrigants facilitate removal of microorganisms, tissue remnants, and dentin chips from the root canal through a flushing mechanism. Amongst other properties, root canal irrigant should have a broad antimicrobial spectrum of activity against anaerobic and facultative micro-organisms growing in biofilms.
and a relative absence of toxicity against periapical tissues and oral mucosa (4).

Biopure MTAD (Dentsply, Tulsa, OK), which is a mixture of a tetracycline isomer, an acid, and a detergent, has been shown to be a clinically effective (5) and biocompatible (6) endodontic irrigant with potential antibacterial substantivity (7). BioPure MTAD has been recommended as a final rinse irrigant because of its antimicrobial properties and its ability to remove the smear layer. It has been reported that MTAD as a final rinse, when used in combination with 1.3% NaOCl as a root canal irrigant, is significantly more effective than 5.25% NaOCl with 17% EDTA in disinfecting root canals contaminated with E. faecalis (8).

Placement of intracanal medicaments such as calcium hydroxide is often recommended for disinfection of the root canal space in teeth with apical periodontitis (9,10). Calcium hydroxide is one of most widely used intracanal medications in endodontics today and remains the best medicament available to reduce residual microbial flora (11). Several worker’s (12) have studied the combination of other substances to calcium hydroxide with the purpose of improving some of its properties. Recently Metapex (Meta Biomed Co. Ltd, Cheongju, Korea), a silicone oil-based calcium hydroxide paste containing 38% iodoform is very popular (13).

Enterococcus faecalis, the most frequently recovered microorganisms from refractory periapical periodontitis, has been used in numerous studies of the antibacterial properties of disinfecting agents because this microorganism has shown resistance to several irrigating solutions and medications used in Endodontics (14). Another bacteria chosen was Propioni bacterium acne (ATCC 6921). Agar disc diffusion test was conducted to evaluate and compare antimicrobial efficacy of medicaments. Bacterial strains were reactivated in Brain Heart Infusion (Difco, Becton-Dickinson and Co., Sparks, MD, USA) broth (BHI) supplemented with hemin (5 mg/l) and menadione (0.5 mg/l) in 48 hours.

Inoculum for each bacterial strain was prepared by picking up four to five colonies with the help of a circular, previously sterilized loop and dissolving them into respective test tube containing 5ml of 0.85% saline solution to produce a turbidity of 0.5 on McFarland scale (1.5 X108 CFU/ml). Sterile cotton swab rolled in the suspension to streak the plate surface of Muller – Hilton – Agar plate (MHA plate) (Oxoid, Cambridge, UK) for Enterococcus faecalis, and brain heart infusion medium (BHI) with 1% glucose for Propionibacterium acne in a form that lawn growth can be observed.

MATERIAL AND METHODS:

The microorganism employed in this study were one anaerobic facultative bacteria, Enterococcus faecalis (ATCC 29212) and one obligate anaerobic bacteria Propionibacterium acne (ATCC 6921).

Data Analysis

Results are expressed as the mean ± one standard deviation. The values were statistically evaluated with one-way ANOVA and the intergroup
comparison was performed with the Bonferroni multiple comparison test. A 'P' value of 0.05 or less was considered as statistically significant. Statistical analysis was performed with SPSS PC version 10 software.

RESULTS

Table 1 shows the mean and standard deviation of Microbial zones of inhibition of tested antimicrobial agents against E.faecalis. Biopure MTAD showed the largest zone of inhibition. Table 2 shows the intergroup comparison of the Microbial zones of inhibition of tested antimicrobial agents against E.faecalis. According to which, there is significant difference in mean zone size of all groups except saline and ethanol which have nonsignificant difference in mean zone size.

<table>
<thead>
<tr>
<th>S.No</th>
<th>M±SD of zones</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTAD</td>
<td>29.25±1.63</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Metapex</td>
<td>26.50±1.23</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.0±0.0</td>
<td>Resistant</td>
</tr>
<tr>
<td>N.Saline</td>
<td>0.0±0.0</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

Table 1: Comparison of the antimicrobial effect of antimicrobial agents against Enterococcus faecalis.

<table>
<thead>
<tr>
<th>Inter group comparison</th>
<th>t value</th>
<th>P value</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1 v Group 2</td>
<td>3.65</td>
<td>&lt;.05</td>
<td>significant</td>
</tr>
<tr>
<td>Group1 v Group 3</td>
<td>38.86</td>
<td>&lt;.001</td>
<td>significant</td>
</tr>
<tr>
<td>Group1 v Group 4</td>
<td>38.86</td>
<td>&lt;.001</td>
<td>significant</td>
</tr>
<tr>
<td>Group2 v Group 3</td>
<td>35.20</td>
<td>&lt;.001</td>
<td>significant</td>
</tr>
<tr>
<td>Group2 v Group 4</td>
<td>35.20</td>
<td>&lt;.001</td>
<td>significant</td>
</tr>
<tr>
<td>Group3 v Group 4</td>
<td>0.0</td>
<td>&gt;.05</td>
<td>Nonsignificant</td>
</tr>
</tbody>
</table>

Table 2: Intergroup comparison of Biopure MTAD (Group-1), Metapex (Group-2), Ethanol (Group-3) and Normal saline (Group-4) against Enterococcus Faecalis.

Table 3 shows the mean and standard deviation of Microbial zones of inhibition of tested antimicrobial agents against P.acnes. Biopure MTAD was most effective against P.acnes. Table 4 shows the intergroup comparison of the Microbial zones of inhibition of tested antimicrobial agents against E.faecalis. According to which, there is nonsignificant difference in mean zone size of Biopure MTAD and Metapex.

<table>
<thead>
<tr>
<th>S.No</th>
<th>M±SD of zones</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTAD</td>
<td>26.00±0.82</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Metapex</td>
<td>24.75±1.02</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.0±0.0</td>
<td>Resistant</td>
</tr>
<tr>
<td>N.Saline</td>
<td>0.0±0.0</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

Table 3: Comparison of the antimicrobial effect of antimicrobial agents against Propionibacterium acne

<table>
<thead>
<tr>
<th>Inter group comparison</th>
<th>t value</th>
<th>P value</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1 v Group 2</td>
<td>2.81</td>
<td>&gt;.05</td>
<td>Nonsignificant</td>
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<tr>
<td>Group1 v Group 3</td>
<td>58.44</td>
<td>&lt;.001</td>
<td>significant</td>
</tr>
<tr>
<td>Group1 v Group 4</td>
<td>58.44</td>
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<td>significant</td>
</tr>
<tr>
<td>Group2 v Group 3</td>
<td>55.63</td>
<td>&lt;.001</td>
<td>significant</td>
</tr>
<tr>
<td>Group2 v Group 4</td>
<td>55.63</td>
<td>&lt;.001</td>
<td>significant</td>
</tr>
<tr>
<td>Group3 v Group 4</td>
<td>0.0</td>
<td>&gt;.05</td>
<td>Nonsignificant</td>
</tr>
</tbody>
</table>

Table 4: Intergroup comparison of Biopure MTAD (Group-1), Metapex (Group-2), Ethanol (Group-3) and Normal saline (Group-4) against Propionibacterium acne.

DISCUSSION

The selection of the test endopathogens for this study was arbitrary and not a random sampling from necrotic teeth. Nevertheless, an attempt was made to select representative bacteria that have been commonly isolated from necrotic canals. The agar diffusion method has been widely in use to test the antimicrobial activity of dental materials and medicaments (16-19). The advantage of this method is that it allows direct comparison of the materials.
against the organisms, indicating the potential of the test material to eliminate bacteria in the local micro environment of the root canal system. However, the disadvantage of this method is that, the result does not depend only on the toxicity of the material for the particular organism, but is also highly influenced by the ability of the material to diffuse across the medium (17,18). E. faecalis is associated with persistent apical inflammation and in clinical situations it is difficult to eliminate from root canal system, therefore selected as a test organism in this study (20,21). In Previous studies Propionibacterium acnes (P.acne) have been identified among the microflora of endodontic infections (22,23). BioPure MTAD, a new irrigant which has 3% Doxycycline (150mg/5ml), 4.25 % citric acid and 0.5% polysorbate80 detergent, was effective against E.faecalis, which support the findings of previous studies (7,24-26) in which they showed the efficacy of BioPure MTAD against E. faecalis.

Biopure MTAD in this study has shown significant zone of inhibition against P.acne. This might be due to presence of doxycycline (broad spectrum antibiotic), as previous literatures (27) have proved the effect of doxycycline on P. acne. Doxycycline is primarily a bacteriostatic antibiotic and inhibits bacterial protein synthesis by binding to the 30S bacterial ribosome. Present study demonstrates that Metapex is effective against E. faecalis, which collaborates with the previous studies (28). Metapex contains silicone oil as its vehicle and has a pH below that which is effective to kill E. faecalis. Oily vehicles increase the antimicrobial effects of Calcium hydroxide against E. faecalis and other bacteria (29). Metapex also shown antimicrobial effect against P.acne, which may be due to the combination with iodoform and to the viscous and oily vehicle, which may prolong the action of the medicament. The strong bactericidal properties of iodoform paste have been demonstrated in previous studies (30,31).

**CONCLUSION**

The results of the present study may be related to the elimination of microorganisms from root canal systems in clinical practice. Under the conditions of this study Biopure MTAD showed better antimicrobial activity then Metapex. According to this work, large scale studies are required to further refine issues related to the effectiveness of various root canal irrigants and intracanal medicaments in endodontology.

**References**


