Research Article

The Antibacterial Efficacy of Biopure MTAD in Root Canal Contaminated with Enterococcus faecalis

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Aim. The purpose of this in vitro study was to assess the antimicrobial efficacy of Biopure MTAD against E. faecalis in contaminated root canals.

Materials and Methods. Forty-two single rooted extracted human teeth were inoculated with E. faecalis and incubated for four weeks. The samples were divided in two control and five experimental groups irrigated with 1.5% sodium hypochlorite solution (NaOCl); 3% NaOCl; BioPure MTAD; 1.5% NaOCl/17% EDTA; or 3% NaOCl/17% EDTA. After a one-week incubation, complete disinfection was confirmed by the absence of turbidity in the incubation media. Dentin shavings were taken from samples with no turbidity to verify whether E. faecalis was present in dentin tubules. Results were analyzed statistically using Fisher’s exact test, with the level of significance set at P<0.05.

Results. Statistical analysis of the data obtained at Day 7 and after dentin shaving analysis showed that BioPure MTAD had significantly greater antibacterial activity than 1.5% NaOCl, 1.5% NaOCl/17% EDTA and 3% NaOCl/17% EDTA. No significant difference was detected between MTAD and 3% NaOCl.

Conclusions. These findings suggest that BioPure MTAD possesses superior bactericidal activity compared with NaOCl and EDTA against E. faecalis.

1. Introduction

The major cause of endodontic failure is the survival of microorganisms in the root-filled tooth. Numerous authors have identified E. faecalis as the predominant microorganism found in root-treated canals displaying persistent periapical disease [1, 2]. The difficulty in eliminating E. faecalis from the root canal is due to its ability to adapt to environmental changes while retaining its pathogenicity [3]. Previous studies report a prevalence of E. faecalis ranging from 24–77% in teeth with failed endodontic treatment [4–8].

Endodontic infections are currently treated by mechanical debridement followed by chemical disinfection. Irrigants are used during the endodontic treatment to flush out loose debris, lubricate the dentinal walls, dissolve organic matter in the canal, and provide antimicrobial activity [9]. Sodium hypochlorite (NaOCl), at concentrations between 0.5–6%, is the most popular irrigating solution due to its antimicrobial activity and its ability to dissolve necrotic tissue [10]. However, sodium hypochlorite does not disinfect the entire root canal system, does not remove the smear layer from the dentinal walls, and is highly destructive when it comes into contact with the periapical tissues and gingiva [11]. In contrast, ethylenediaminetetraacetic acid (EDTA) has low or no antibacterial activity, but effectively removes the smear layer by affecting the inorganic component of the dentine. By facilitating the removal of infected tissue, EDTA contributes to the elimination of bacteria in the root canal [12]. Thus, to facilitate root canal disinfection, it is recommended that an irrigant containing both NaOCl and EDTA be used [11].

Torabinejad et al. [13] have reported the development of new irrigants for use in canal disinfection and smear layer removal, including BioPure MTAD (Dentsply, Tulsa, OK), a mixture of a tetracycline isomer [doxycycline], an acid [citric acid], and a detergent [Tween 80]. The doxycycline present
in MTAD has high binding affinity for dentine, allowing for a prolonged antibacterial effect [14]. BioPure MTAD has been recommended as a final rinse irrigant because of its antimicrobial properties and its ability to remove the smear layer [11, 13, 15–17]. It is also less cytotoxic than most endodontic medicaments, including eugenol, hydrogen peroxide (3%), EDTA, and calcium hydroxide paste [15–18]. However, it has also been reported that BioPure MTAD (Dentsply) may not be effective against E. faecalis [19–21]. Therefore, the purpose of this study was to assess the antimicrobial efficacy of BioPure MTAD against E. faecalis compared to conventional endodontic irrigants.

2. Materials and Methods

The methodology used in the present study was modified slightly from that described previously by Shabahang and Torabinejad [16]. Forty-two single-rooted extracted human teeth were used for this study. Samples were stored in water to avoid dehydration before use. After gaining access, the pulp was removed, irrigated with distilled water and all teeth were sterilized in an autoclave at 121°C (Sterilizatoren GmbH, Oiching, Germany).

Pure cultures of E. faecalis (ATCC 29212 OXOID, Hampshire, UK) were incubated overnight in blood heart infusion broth (BHI-Oxoid LTD., Wade Road, Basingstoke, Hampshire, UK) with a preparation of 1 × 10⁸ bacteria in 125 mL broth sufficient to prepare five experimental tubes. Teeth were immersed in inoculum and incubated at 37°C for four weeks in aerobic conditions in an incubator (INNOVENS 53, Jouan, France). This incubation period was sufficient for E. faecalis to invade the dentinal tubules. Culture media was refreshed every third day to maintain bacteria levels. Each tooth was sampled with paper points, from external and internal surface, inoculated on BHI plates to confirm the presence of infection.

Following four weeks of infection, samples were divided into seven groups: two control and five experimental groups. Working length was established by using #10 K-file to penetrate the apical foramen and then pulled back 1 mm. The teeth were manually instrumented with Flexo Files (Dentsply, Mailfeller Ballaigues, Switzerland) up to size #40 using a passive step-back technique. During cleaning and shaping, rigorous aseptic techniques were followed using sterile gloves and pliers. In the positive control group, irrigation was performed using distilled water. In the negative control group, the irrigant was also distilled water, but irrigation was followed by a period of autoclaving. Sterilization of these samples ensured that contamination of the working field did not occur. The remaining teeth were divided into five experimental groups, as detailed in Table 1.

Following preparation, each tooth was immersed in 2 mL of BHI and vortexed for 15 s. Teeth were then transferred into tubes containing fresh BHI and incubated for one week at 37°C under aerobic conditions. Complete disinfection was confirmed by the absence of media turbidity at one-week period. Samples that showed turbidity were classified as infected, and the presence of bacteria was identified on BHI agar plates, and observed with microscope to identify Gram-positive cocci in chains.

To determine the presence of bacteria in the dentinal tubule, dentin shavings were taken with sterile carbide bur no. 3. Shavings were taken from those samples that exhibited no turbidity to verify whether E. faecalis was present in the dentinal tubules. The teeth and dentin shavings were cultivated to determine the presence or absence of E. faecalis. Turbidity in cultivated solutions indicated the presence of E. faecalis. Shavings were collected on BHI agar plates and incubated for 48 h.

Results were analyzed by Fisher’s exact test, with the level of significance set at P < 0.05.

3. Results

Turbidity was evident in all of the positive control samples, but in none of the negative controls. The presence of turbidity in test samples is summarized in Table 2. After one week of incubation, turbidity was evident to some degree in each of the groups treated with NaOCl or NaOCl + EDTA. In contrast, none of the samples treated with BioPure MTAD were visibly infected. When dentine shavings were incubated in BHI broth, a single sample in each of the five groups was infected with E. faecalis.

Statistical analysis of the total number of infected samples in each group using Fisher’s exact test showed significant differences between BioPure MTAD and 1.5% NaOCl, 1.5% NaOCl/17% EDTA and 3% NaOCl/17% EDTA (P = 0.008 for each comparison), but no significant difference between BioPure MTAD and 3% NaOCl (P = 0.242). Because each group had a single sample (out of six) found to be infected in the dentinal tubules, this difference can be attributed entirely to differences in the rate of infection after the one-week incubation. Indeed, Fisher’s exact test found near-identical results when analyzing these rates specifically (P = 0.008 for BioPure MTAD versus 1.5% NaOCl, 1.5% NaOCl/17% EDTA; 3% NaOCl/17% EDTA; P = 0.227 for BioPure MTAD versus 3% NaOCl).

4. Discussion

Enterococcus faecalis is commonly found in failed root-treated canals [22], due mainly to its resistance to chemomechanical procedures [6] and intracanal medication such as calcium hydroxide [23]. In our experiments, positive control samples showed that distilled water is totally ineffective at eliminating E. faecalis. Furthermore, negative control samples confirmed that the incubation environment used during the experiment was not contaminated. We chose several irrigants to evaluate their efficacy in eliminating E. faecalis from the root canal system. NaOCl, in addition to its excellent anti-bacterial properties, is known to be cytotoxic at higher concentrations, so we evaluated this irrigant at both low (1.5%) and high (3%) concentrations. Our results demonstrate that irrigation with NaOCl, even at the high concentration, eliminated E. faecalis in only half of the samples. This lack of efficacy of NaOCl in consistently
exhibited less antimicrobial efficacy against E. faecalis. Newberry et al. [26] determined the antimicrobial effect of BioPure MTAD as a root canal irrigant against E. faecalis. In another study, Mohammadi and Shahriari [28] compared the antimicrobial efficacy of irrigation with 1.3% NaOCl/Biopure MTAD with that of irrigation with 5.25% NaOCl/15% EDTA in the apical 5 mm of roots infected with E. faecalis, and found no difference between these treatments. In addition, Baumgartner et al. [21] found no growth of E. faecalis in root canals irrigated with 5.25% NaOCl/15% EDTA, while 50% of the canals irrigated with 1.3% NaOCl/Biopure MTAD demonstrated growth of E. faecalis, a difference that was statistically significant. Our results are also in disagreement with those of Dunavant et al. [19], Giardino et al. [33], Clegg et al. [34], Ruff et al. [35], and Krause et al. [36], each of whom showed that NaOCl was more effective than BioPure MTAD at eliminating E. faecalis. These differences ensure that the efficacy of BioPure MTAD remains somewhat controversial, although are probably in part explained by methodological differences such as alternative microbial sampling procedures or deviation from the manufacturer’s usage recommendations when using BioPure MTAD.

5. Conclusions

Our findings suggest that BioPure MTAD possesses superior bactericidal activity compared with NaOCl and EDTA against E. faecalis in contaminated root canals. However, further clinical studies are required to confirm the in vivo antimicrobial effects of this and other endodontic medicaments.

Disclosure

This paper is approved by all the coauthors. Also, this paper has the approval of the institution.
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