**ABSTRACT**

**Background and Aim:** The purpose of this study was to evaluate the antimicrobial effects of 2% sodium hypochlorite (NaOCl), 2% chlorhexidine gluconate (CHX), 2.4% iodine potassium iodide (IKI), 17% ethylenediaminetetraacetic acid (EDTA) and bioactive glass (BAG) on four selected microorganisms (E. faecalis, E. coli, S. aureus, S. pyogenes).

**Materials and Methods:** Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined in an incubation period of 24 hours for the selected medicaments on standard bacterial strains using the microdilution method.

**Results:** MIC/MBC values of EDTA for S. aureus and S. pyogenes were both 0.066% (dilution factor of 1/256). The MIC/MBC values of EDTA for E. faecalis were 0.033% (1/512) and 0.13% (1/128). The MIC dilution factor of EDTA was equal/two-fold higher for IKI and NaOCl for E. coli, 8-fold higher for S. pyogenes and 16-fold higher for S. aureus and E. faecalis within clinically relevant concentrations of the test irrigants. All microorganisms were resistant to BAG and CHX.

**Conclusion:** The results of this study indicate that EDTA exhibits strong in vitro antimicrobial effect against S. aureus, S. pyogenes and E. faecalis in 24 hours. NaOCl displayed activity equivalent to that of IKI on E. coli, S. aureus and E. faecalis.
Clinical Dentistry and Research

Introduction

The elimination of microorganisms and their by-products from an infected root canal system is essential for a successful endodontic treatment. Reducing the bacterial count in infected root canals is accomplished with a combination of mechanical instrumentation, various irrigation solutions, and antibacterial medicaments or dressings placed into the canal. However, infection may persist in canals with anatomic complexities, with a great number of bacteria. Numerous studies have shown that the bacterial flora in endodontic infections is polymicrobial with dominance of anaerobic species. Using the advanced anaerobic bacteriological techniques, Lana et al. showed that a polymicrobial environment existed in necrotic teeth, consisting of obligate and facultative anaerobes, microaerophilic bacteria and yeasts. Elimination of these microorganisms in the root canal space is essential in preventing them from reaching the periapical tissues. The ideal irrigant should be strongly antimicrobial, but not toxic to the periapical tissues if extruded through the apex. Sodium hypochlorite (NaOCl) has been widely recommended as an irrigant for its antimicrobial and tissue dissolving properties. However, there is concern about its potential toxic effect on the periapical tissues at higher concentrations. At lower concentrations, the tissue dissolving ability and antimicrobial effectiveness are reduced. The antimicrobial ability of NaOCl results from the formation of hypochlorous acid (HOCl), when in contact with organic debris. HOCl exerts its effect by the oxidation of sulfhydryl groups of bacterial enzymes, cleaving disulfide bonds. However, it kills rapidly and has bactericidal, fungicidal, tuberculocidal, virucidal, and even sporicidal activity. Bioactive glasses (BAG) have the ability to mineralize dentin and also have an antimicrobial effect. The short-term antimicrobial effect of these glasses has been attributed exclusively to their ability to raise pH in an aqueous environment.

In the literature, the number of researches related to the use of BAG as an intracanal irrigant is limited. Research on efficient irrigating concept to cover the wide range of microorganisms is in progress. Therefore, the purpose of this study was to evaluate the in vitro antimicrobial activities of BAG, 2% NaOCl, 2% CHX, 2.4% IKI, and 17% EDTA on Enterococcus faecalis, Escherichia coli, Staphylococcus aureus, and Streptococcus pyogenes.

Materials and Methods

Bacterial strains

Standard strains representing each microorganism evaluated in this study consisted of Enterococcus faecalis (ATCC 29212), Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 29213), and Streptococcus pyogenes (ATCC 19615). Prior to the study, stock bacterial strains kept at -80°C were cultivated on brain heart infusion (BHI) agar (supplemented with 5% sheep blood for S. pyogenes) at 37°C for 24 hours.

Preparation of test materials

2.5% NaOCl (ACE, Procter & Gamble, Istanbul, Turkey), 2.4% IKI, 2% CHX (Drogsan, Ankara, Turkey), and 17% EDTA, BAG powder S53P4 (Abmin Technologies Ltd, Turku, Finland) were used in this study as the test materials. The testing concentrations were within the range recommended for clinical use in root canal treatment. The glass powder was sieved to a particle size of ≤ 45 µm, with mean diameter of approximately 20 µm. BAG was suspended in unbuffered physiologic saline (0.9% NaCl) at 37°C under constant stirring. The solid-to-liquid ratio of the suspension was 1:2. IKI was prepared by mixing 2 g of iodine in 4 g of potassium iodide; this mixture is then dissolved in 94 ml of distilled water.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC determinations were performed using 96-
well plates with standard protocols. Briefly, the numbers of each bacterial strain grown on solid media were adjusted nephelometrically to 0.5 McFarland standard (∼1.5 x 10⁸ colony forming units [cfu]/ml of bacteria), and 1/10 and 1/100 dilutions were prepared in Mueller-Hinton broth. One hundred microliters of bacterial dilutions were transferred to each well and equal amounts of medicaments were prepared in wells to establish two-fold serial dilutions, ranging from 1/2 to 1/512. Medicaments and bacteria diluted in 1/2 in Mueller-Hinton broth were used as negative and positive controls, respectively. After 24-hour incubation at 35°C, the lowest dilution of each medicament preventing the appearance of visible turbidity in the well was considered as the MIC. For the determination of MBC, 100 µl from the wells in which no turbidity was observed were subcultured onto blood agar plates and incubated at 35°C for another 24 hours. The dilution of medicament where no colony formation was observed on the plates was determined as the MBC. All experiments were duplicated.

RESULTS

The MIC and MBC values obtained for each bacterium are reported in Table 1. These are expressed as both a percentage ratio according to the stock solution and in fold dilutions (Table 1).

While IKI displayed MIC/MBC values for E. coli (0.037%, 1/64) equal to those of EDTA, the observed MIC/MBC values for EDTA for S. aureus and S. pyogenes were 0.066% (1/256). The MIC/MBC values of EDTA for E. faecalis were observed to be 0.033% (1/512) and 0.13% (1/128), respectively. The results of this study indicated EDTA to be the most efficient medicament overall. NaOCl displayed activity equivalent to that of IKI on S. pyogenes, S. aureus and E. faecalis. CHX and BAG were not found to be effective at the test dilutions of 2.4% and 10%, respectively, in an incubation period of 24 hours (Table 1).

For all medicaments tested for each bacterium, the MIC values were equal or one dilution lower than the MBCs, indicating that each chemical acts as an effective disinfectant, once the inhibitory concentrations are reached in the root canal.

DISCUSSION

It is important to eliminate most of the bacteria species from the root canal system during the first canal appointment using efficient irrigant and to prevent re-contamination by providing adequate coronal seal.

The microorganisms tested in this study are part of the endodontic microbial flora. Concentrations of the tested

<table>
<thead>
<tr>
<th>Materials</th>
<th>E. coli</th>
<th>S. pyogenes</th>
<th>S. aureus</th>
<th>E. faecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>NaOCl (stock: 2.5%)</td>
<td>0.078% (1/32)</td>
<td>0.078% (1/32)</td>
<td>0.156% (1/16)</td>
<td>0.156% (1/16)</td>
</tr>
<tr>
<td>IKI (stock: 2.5%)</td>
<td>0.037% (1/64)</td>
<td>0.037% (1/64)</td>
<td>0.15% (1/16)</td>
<td>0.3% (1/8)</td>
</tr>
<tr>
<td>CHX (stock: 2.4%)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>BAG (stock: 10%)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>EDTA (stock: 17%)</td>
<td>0.26% (1/64)</td>
<td>0.26% (1/64)</td>
<td>0.066% (1/256)</td>
<td>0.066% (1/256)</td>
</tr>
</tbody>
</table>

R: resistant to all dilutions tested in the study
materials used in this study were within the range recommended for clinical use in root canal treatment. The microdilution method is the standard in vitro method for determining MICs and MBCs for most of the bacterial strains; thus, this was the method of choice for this study. The MICs and MBCs obtained for each bacterium are reported in Table 1. The observed MICs and MBCs were in agreement with the results for most antimicrobial agents for each bacterium tested separately.

EDTA and its commercial preparations (EDTAC, RC-Prep) have long been used as irrigants in endodontics. EDTA has a germicidal effect at a concentration of 10% as reported by Patterson. Vineeta et al. showed that EDTA (10% and 15%) has antimicrobial activity, both on culture plates and in broth. A comparison of bacterial growth inhibition showed that the antimicrobial effect of EDTA was stronger than that of citric acid and 0.5% NaOCl but weaker than 2.5% NaOCl and 0.2% CHX. In this study, the MIC/MBC values showed that EDTA inhibited E. faecalis even when diluted 512 times (0.033% concentration). The determined MIC/MBC dilutions were observed as 1:256 for S. aureus and S. pyogenes and 1:64 for E. coli. The MIC dilution factor of EDTA was equal/two-fold higher for IKI and NaOCl for E. coli, 8-fold higher for S. pyogenes and 16-fold higher for S. aureus and E. faecalis within clinically relevant concentrations. In the light of our findings, it is evident that EDTA displays considerable in vitro antimicrobial effects on selected microorganisms in an incubation period of 24 hours.

While NaOCl was more effective in dilutions of 1:32 on E. coli, S. aureus and E. faecalis and in 1:16 dilution on S. pyogenes, IKI inhibited E. coli when diluted 64 times, and the MIC dilution factors were 1:32 for S. aureus and E. faecalis and 1:16 for S. pyogenes. These results suggest equivalent activity of IKI and NaOCl, except that IKI is more effective on E. coli and with two-fold higher MBC for S. pyogenes (Table 1).

CHX is not effective against E. coli, E. faecalis, S. aureus and S. pyogenes at the aforementioned test concentrations and incubation period. CHX has a cationic molecular component that attaches to negatively charged cell membrane areas, causing cell lysis. It has substantivity and long-lasting antimicrobial effect, which arises from binding to hydroxyapatite. Therefore, antimicrobial efficacy studies of prolonged duration are required to determine its effective action against microorganisms. It has been shown in chemical analyses that chlorine, which is the active agent in NaOCl, is inactivated by EDTA. It is suggested that since the effect of lubricants containing EDTA on rotary instrument torque is unproven, use of these solutions probably should be limited to hand instrumentation early in a procedure. Recent reports have indicated that several disinfecting agents such as Ca(OH)₂, IKI and CHX are inhibited in the presence of dentin. The buffering of dentin is one of the obstacles to the success of root canal therapy, so this needs to be taken into consideration in further research. In one study, the glass used was composed of 53% SiO₂ (w/w), 23% Na₂O, 20% CaO, and 4% P₂O₅ and was prepared from reagent grade Na₂CO₃, CaHPO₄.2H₂O, CaCO₃ and Belgian sand. When used in root canals, BAG was found to kill bacteria, but the mechanism of action was not pH-related and dentin did not seem to alter its effect. Further research is needed for determining if similar antimicrobial activities will be obtained with a more clinically relevant model.

CONCLUSION

All microorganisms investigated in this study were sensitive to NaOCl, IKI and EDTA. EDTA was the most effective irrigant for initial (in 24 hours) elimination of the bacteria among those three irrigants. Based on the results of this study, we can suggest that EDTA can be used as an antimicrobial agent and in addition for its lubricant effect at the initial appointment. Thus, during the shaping procedure of the canal, interactions between EDTA and NaOCl that reduce the antimicrobial effect of NaOCl can be eliminated. All microorganisms were more resistant to CHX and BAG in 24 hours.

ACKNOWLEDGEMENT

This study received a grant from the Hacettepe University Research Fund (project number 07D01201002), and was presented at the 4th Scientific Symposium of the Endodontic Societies of Turkey and Kosova, 23-26 April 2009, Antalya, Side, Turkey.

REFERENCES


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