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Antimicrobial activity of different disinfectants against cariogenic microorganisms

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Abstract: The aim of this study was to assess the *in vitro* antimicrobial effects of chlorhexidine digluconate (CHX), polyhexamethylene biguanide (PHBM), and octenidine dihydrochloride (OCT) on cariogenic microorganisms by using their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). CHX, PHBM, and OCT were diluted in distilled water to the final test concentrations. Using the in-tube dilution method, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Actinomyces viscosus* were cultivated on blood agar and Mueller–Hinton broth (MHB) at 37°C for 48 h. They were read using a spectrophotometer to detect MIC. To determine MBC, samples in the range of the turbidity threshold after 24 h were transferred onto blood agar and evaluated for growth after 24 h. Different MICs and MBCs were observed in all disinfectants against each microorganism. The lowest MIC and MBC against *S. mutans* (60 mg/L) were obtained from PHBM. The lowest values against *L. rhamnosus* (15 mg/L, 30 mg/L), *A. viscosus* (30 mg/L), and *L. acidophilus* (15 mg/L, 30 mg/L) were determined by OCT. PHBM and OCT have the potential to be replaced with CHX because they were effective against cariogenic microorganisms.

Keywords: Octenidine; Polyhexamethylene Biguanide; Chlorhexidine Gluconate.

Introduction

Dental caries is a localized and transmissible pathological infectious process that results in the destruction of hard dental tissues.[1] The elimination of cariogenic microorganisms is one of the crucial factors for the prevention of primary or residual caries lesions.[2] Recent research shows that *Streptococcus mutans*, *Lactobacillus acidophilus*, *Actinomyces viscosus*, and *L. rhamnosus* are the most common cariogenic microorganisms.[3,4,5] *S. mutans* is the main etiological agent in dental caries.[6] It is associated with the initiation of caries and is also isolated both from enamel carious lesions and hidden dentin caries.[7] *Lactobacillus* is a group of bacteria that occurs at high levels in deep dentin caries.[8] The analysis shows that among the genus *Lactobacillus*, *L. acidophilus* is most prevalent, and *L. paracasei*, *L. rhamnosus*, and *L. fermentum* are also present in deep caries lesions and promote caries progression.[8,9] Furthermore, the fact that *L. rhamnosus* constitutes the dominant species in carious dentin both in deciduous and permanent teeth has been supported

by numerous authors using biochemical or reliable genetically-based methods.[2,10,11,12,13,14] In addition to *Lactobacillus* species, anaerobic conditions have resulted in the isolation of *Actinomyces* species from dentin lesions.[15]

To reduce the potential of primary or residual caries and enhance the postoperative sensitivity, antibacterial agents may be chosen according to their ability to reduce or eliminate the possibility of existing bacteria. Thus, the use of antimicrobial solutions as an oral rinse or a cavity disinfectant for reducing cariogenic microorganisms according to the target of the application has been recommended. Chlorhexidine digluconate (CHX) is a commonly used antimicrobial agent because of its ability to significantly reduce the levels of cariogenic microorganisms.[16-18]

However, CHX had adverse effects, including staining of the teeth and tongue, mucosal soreness, and desquamation; temporary taste disturbances; hypersensitivity; and selective disturbance of the microbial balance within the oral ecosystem.[19] Previous studies have shown that CHX was cytotoxic to human fibroblasts via the inhibition of protein synthesis.[20] In addition, the resistance of *L. rhamnosus* to CHX therapy was also reported.[21] Therefore, antimicrobial agents, such as octenidine dihydrochloride (OCT) and polyhexamethylene biguanide (PHMB) have been investigated as alternatives to CHX because of their superior microbicidal activity and lower cytotoxicity than CHX.[22,23,24,25]

OCT is an antiseptic agent recently used for the management of skin burns, wounds, and as a mouth rinse. OCT belongs to the bipyridines, carrying two cationic active centers per molecule, and demonstrates the broad-spectrum antimicrobial effects on both gram-positive and gram-negative bacteria, fungi, and several viral species.[26] The bactericidal and fungicidal effects are primarily achieved by interfering with the cell wall and membranes of such microbes. Previous studies have shown that the efficacy of OCT against dental plaque-associated bacteria (*e.g.*, *S. mutans* and *A. viscosus*) was comparable to that of CHX.[24,27]

PHMB is a type of modern antiseptic that combines a broad antimicrobial spectrum with low toxicity, high tissue compatibility, no reported adsorption, and is used as a solution, gel, ointment, or foam. As such, it is one of the most promising antiseptic substances.[25]

Although some studies have been published concerning the antimicrobial properties of OCT on *S. mutans* and *A. viscosus* as well as that of PHMB on *S. mutans*,[28,29] there is a lack of studies regarding their antibacterial activity on other essential cariogenic microorganisms (*e.g.*, *L. rhamnosus* and *L. acidophilus*). Therefore, the aim of the present study was to assess the *in vitro* antimicrobial effects of CHX, OCT, and PHMB on cariogenic microorganisms using both their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Methodology

The experiments were performed at the Microbiology Laboratory in the Department of Biology, Faculty of Science, Ege University. Culture media brain-heart infusion (BHI) (Difco, Detroit, MI, USA) broth and agar were made. The tubes and the plates were sterilized in an autoclave, tested for sterility, and kept under refrigeration until use.

CHX (Drogsan, Ankara, Turkey), PHMG (Mertsel Ilac, Izmir, Turkey), and OCT (Schülke & Mayr GmbH, Norderstedt, Germany) were obtained as solutions and diluted in water of standardized hardness (WSH, according to DIN EN 1040) to the test concentrations. The initial concentrations of OCT and PHMB were 0.2% (2,000 mg/L), and 2% (20,000 mg/L) for CHX. The tube dilution method was used for determining MIC and MBC of the antimicrobial agents against the selected microorganisms.

In this study, *S. mutans* (ATCC 25175), *L. acidophilus* (ATCC 4356), *A. viscosus* (ATCC 15987), and *L. rhamnosus* (ATCC 7469) were used; all were previously frozen. All bacterial strains were obtained from the American-type culture collection.

The microorganisms were reactivated in sterile BHI broth and incubated at 37°C in a carbon dioxide incubator (SANYO Electric Biomedical Co., Ltd., Osaka, Japan) at 5% CO_2. After 48 h of culture growth, the Gram staining was used and the cultures were replated to verify their purity. The microorganisms were transferred to the broth media and their absorbance indexes were verified hourly using

a spectrophotometer at a wavelength of 540 nm. Bacterial suspensions equal to the No. 0.5 McFarland standard were prepared.

Ten sterile test tubes were collected and 1 mL of sterile tryptic soy broth (TSB) was added to each tube. Then, 1 mL of each solution with specified dilutions was prepared using the serial dilution (1:2) method and added to each of the tubes. A bacterial suspension of 1.5×10^8 CFU equal to the No. 0.5 McFarland standard was prepared from the standard strains.

A 1 mL volume of the dilute suspension was added to each set of eight tubes that contained the TSB medium and solution. After the bacterial suspension was added to the test tubes, they were placed in a carbon dioxide incubator (to provide 5% CO_2) and were incubated at 37°C for 24–48 h. After this period had elapsed, the tubes were examined for the presence of turbidity, which indicated microbial growth. The last tube, or the last dilution of solutions at which turbidity was not observed, was considered MIC of the respective solutions for certain microorganisms. MIC of each of the three solutions was then compared for the ability to inhibit microbial growth. After a 24 h incubation, the tubes without turbidity (transparent), which indicated the inhibition of bacterial growth by the respective solutions, were transferred to a solid medium (TSA agar) and evaluated for microbial growth to determine the MBC of the solutions. The last tube, which was negative regarding the presence of cultures on a solid medium, indicated MBC of the solutions. This procedure was performed for all bacterial strains. The experiment was performed in triplicate.

Results

All disinfectants tested had bactericidal and bacteriostatic effects on the cariogenic microorganisms tested in this study. However, different MICs and MBCs were observed in all disinfectants against each microorganism (Figure).

The lowest MIC values against *S. mutans* were obtained from CHX (70 mg/L) and PHBM (60 mg/L), while MIC of OCT (120 mg/L) was two times greater than that of CHX and PHBM. The lowest MBC was obtained from PHBM (60 mg/L), while MBC of CHX (150 mg/L) and OCT (120 mg/L) was nearly twice that of PHBM.

The lowest MIC against *L. rhamnosus* was achieved by OCT and PHBM (15 mg/L), while MIC of CHX (150 mg/L) was ten times greater than OCT and PHBM. The lowest MBC was determined by OCT and PHBM (30 mg/L), while MBC of CHX (150 mg/L) was five times greater than OCT and PHBM.

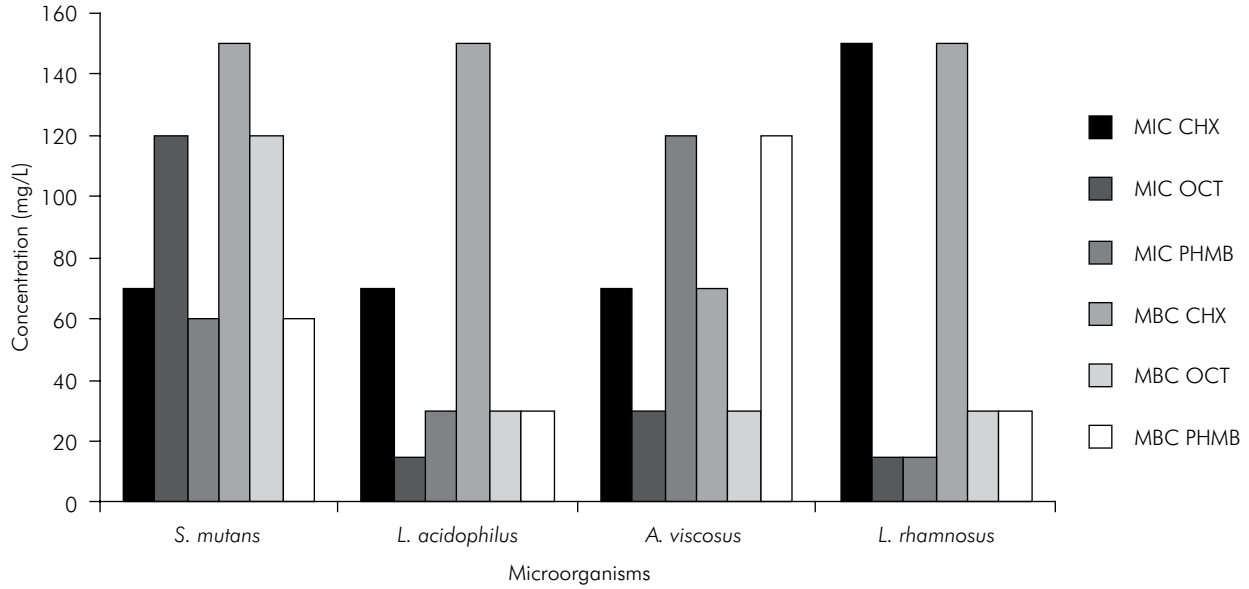

Figure. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of various antimicrobial agents.

The lowest MIC against *L. acidophilus* was found to be OCT (15 mg/L), while MIC of PHMB (30 mg/L) was two times and MIC of CHX (70 mg/L) was nearly four times greater than OCT. The lowest MBC was obtained from OCT and PHMB (30 mg/L), while MBC of CHX (150 mg/L) was five times greater than OCT and PHMB.

The lowest MIC and MBC against *A. viscosus* (30 mg/L) was determined by OCT, while MIC and MBC of CHX (70 mg/L) were two times and MIC and MBC of PHBM (120 mg/L) were four times greater than OCT.

Discussion

MICs and MBCs of the antimicrobial agents investigated in the present study were determined using the microdilution test method. MICs are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after an overnight incubation and are considered the gold standard for determining the susceptibility of organisms to antimicrobials. On the other hand, MBCs are the lowest concentrations of an antimicrobial that will prevent the growth of an organism following a subculture on antimicrobial-free media.[30] MBCs are used for determining the potential resistance of an antimicrobial and making rational decisions in determining how successful an antimicrobial treatment is likely to be.[30] However, MBC is more relevant than MIC for assessing the antibacterial activity of a solution and is also higher than MIC.[31] In this study, the initial concentration of the tested solutions were different and are the commonly used concentrations for these solutions.

Three commonly used antimicrobial agents were tested. CHX is used at a concentration of 0.12% and 0.2% as oral rinses, and 2% as an endodontic irrigation solution and cavity disinfectant.[32] Additionally, for CHX to be used as an anticarcinogenic agent, a 1% concentration in a gel formulation is available.[33,34] OCT is commonly used at a concentration of 0.1%–0.2% and PHMB is used at 0.2% as an antiseptic.[25,35,36]

It was demonstrated that all antiseptic solutions had both bacteriostatic and bactericidal effects against the test microorganisms, as CHX was used at 150 mg/L and OCT and PHMB were used at 120 mg/L in the present study. All of the bacterial groups that were chosen as the test microorganisms in this study were the most common cariogenic bacteria isolated from caries lesions.[3,4,5,7,8,9,15] The antimicrobial effects of these agents against oral microorganisms, such as *S. mutans, S. sanguinis*, *C. a1lbicans*, and *F. nucleatum* were previously confirmed by Kocak et al.[30] and Rohrer et al.[37] However, the antibacterial effects of these agents on the other crucial microorganisms in caries progression (*e.g.*, *L. rhamnosus* and *L. acidophilus*) were not assessed. The most important findings in the present study included the effectiveness of these antimicrobials against common cariogenic bacteria and MIC of these agents.

PHMB and CHX were both effective against all microorganisms tested; however, against *Lactobacillus* species and *S. mutans*, PHMB was effective at the same or slightly lower concentrations than CHX. This finding is in accordance with Rohrer et al.[37] who stated that the antimicrobial activity of PHMB was comparable to that of CHX against *S. mutans* and *S. sanguinis*. PHMB is a biguanide with effective antibacterial properties. The antibacterial effect is mediated by an increase in the permeability of the bacterial cell membrane, which leads to an osmotic imbalance and an outpouring of cytoplasm. Polyhexanides have a broad antibacterial spectrum, mainly against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Enterobacter cloacae*, and *Streptococcus lactis*. In addition to its effective antibacterial activity against the cariogenic microorganisms tested, the cytotoxicity of PHMB is low, and thus, the tissue compatibility is high. The local tolerability of PHMB is superior compared to other disinfectants, such as iodine, hydrogen peroxide, or CHX.[38]

In the present study, OCT was found to be more effective than CHX at lower concentrations against each microorganism. Similarly, Kocak et al.[30] and Dogan et al.[38] found in their previous studies that OCT was more favorable than CHX regarding its antibacterial activity against *S. mutans* and *Lactobacillus* species, both *in vitro* and *in vivo*. OCT was originally developed as a potential broad-spectrum topical antimicrobial agent,[39] and its use as an oral rinse has been reported to inhibit the development of dental plaque in both primates[27] and in humans.[40,41] One recent study

showed that a 0.1% OCT mouth rinse provided a statistically significant reduction of 39% of plaque, 50% of gingivitis, and 60% of gingival bleeding sites.[42] Moreover, in an *in vivo* study, it was observed that 0.1% OCT had a significant effect on *S. mutans* in both 1 and 10 min time periods, and the antimicrobial efficiency was preserved even after 60 min.[29]

CHX, OCT, and PHMB were found to be effective against the most common cariogenic microorganisms in this study. However, their potential use in clinical procedures and their effect on biofilm formation, bacterial adhesion, and bond strength of adhesive systems should be investigated with future *in vitro* and *in vivo* studies.

Conclusion

The findings of the present study suggest that OCT and PHMB are significantly effective against cariogenic microorganisms at 120 mg/L concentrations. OCT and PHMB can be considered potential alternatives to CHX for reducing cariogenic microorganisms during antibacterial procedures.

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