Antimicrobial Efficacy of 3 Oral Antiseptics Containing Octenidine, Polyhexamethylene Biguanide, or Citroxx: Can Chlorhexidine Be Replaced?

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BACKGROUND. Use of oral antiseptics decreases the bacterial load in the oral cavity.

OBJECTIVE. To compare the antimicrobial activity of 3 novel oral antiseptics with that of chlorhexidine, which is considered the “gold standard” of oral hygiene.

DESIGN. Comparative in vitro study.

METHODS. Four common oral microorganisms (Streptococcus sanguinis, Streptococcus mutans, Candida albicans, and Fusobacterium nucleatum) were tested under standard conditions and at different concentrations, by use of a broth dilution assay and an agar diffusion assay and by calculating the log_{10} reduction factor (RF). The antimicrobial activity of each antiseptic was assessed by counting the difference in bacterial densities (ie, the log_{10} number of colony-forming units of bacteria) before and after the disinfection process.

RESULTS. The oral antiseptics containing octenidine (with an RF in the range of 7.1–8.24 CFU/mL) and polyhexamethylene biguanide (with an RF in the range of 7.1–8.24 CFU/mL) demonstrated antimicrobial activity comparable to that of chlorhexidine (with an RF in the range of 1.03–8.24 CFU/mL), whereas the mouth rinse containing Citroxx (Citroxx Biosciences; with an RF in the range of 0.22–1.36 CFU/mL) showed significantly weaker antimicrobial efficacy. Overall, octenidine and polyhexamethylene biguanide were more active at lower concentrations.

CONCLUSION. Oral antiseptics containing the antimicrobial agent octenidine or polyhexamethylene biguanide may be considered as potent alternatives to chlorhexidine-based preparations.

The mouth and oropharynx are colonized with microorganisms, which include gram-negative anaerobic bacteria, Staphylococcus aureus, and Candida species. The most common oral infections associated with bacteria are diseases of the tooth-supporting structures (ie, gingivitis and periodontitis), affecting up to 98% of adults in the United States. In addition, these microorganisms have the ability to invade the bloodstream, resulting in transient bacteremia, especially during tooth brushing and flossing (20%–68% of cases) and even during the chewing of food (7%–51% of cases). The microorganisms found in the oral cavity are also associated with pneumonia. A meta-analysis by Chan et al provided indirect evidence of the impact of oral microorganisms; they showed that oral decontamination with an antiseptic administered as prophylaxis reduces the incidence of ventilator-associated pneumonia. Similarly, a randomized controlled clinical trial showed that selective oral decontamination resulted in a significant reduction in cases of ventilator-associated pneumonia.

Chlorhexidine is a cationic biguanide that was introduced as an antimicrobial agent by G. E. Davies in 1954. Because of the antimicrobial spectrum and the suspected remnant effect, chlorhexidine digluconate is considered the “gold standard” of oral hygiene in the United States. However, the use of chlorhexidine-containing mouth rinses can result in adverse effects, such as taste disturbance or tooth staining, that may have an impact on compliance. In addition to the cytotoxicity of chlorhexidine on corneal and endothelial cells, the neurotoxicity of chlorhexidine was observed in animal models, which prevents it from being used for very long. Allergic reactions are common if chlorhexidine is applied in concentrations of more than 4%, and serious anaphylactic reactions have been described. A higher concentration of chlorhexidine would be preferable for mouth rinses because...
they are diluted after application. However, there is evidence that the antimicrobial effect of chlorhexidine is primarily bacteriostatic, rather than bactericidal, and that the antimicrobial effect of chlorhexidine is probably overestimated because of lack of neutralization after sampling. In addition, its efficacy against several gram-negative pathogens is limited.

In Europe, additional oral antimicrobial mouth rinses containing octenidine, polyhexamethylene biguanide, or Citrox (Citrox Biosciences) have been marketed that claim to have a similar antimicrobial spectrum but are associated with fewer adverse effects, less toxicity, and less frequency of allergic reactions. Citrox consists of a patented blend of bioflavonoids and fruit acids. Citrox-containing solutions are frequently used as active ingredients in a variety of applications, cosmetics, personal care products, or hand washes.

In addition to polyhexamethylene biguanide’s antibacterial activity against oral microorganisms, it has been used in antimicrobial gauze dressing to prevent surgical site infections due to methicillin-resistant S. aureus. Octenidine may be used for prolonged periods of time because it is associated with fewer adverse effects, compared with chlorhexidine. In the clinical setting, octenidine has been successfully used for the prevention of catheter-related infections.

A standardized follow-up period, which included laboratory analysis of sessile and planktonic bacteria, was suggested for the evaluation of the antimicrobial activities of oral antiseptics. Finally, the therapeutic efficacy of each antiseptic has to be evaluated in clinical studies (ie, with the use of phase 3 tests).

However, to our knowledge, there have been no side-by-side in vitro studies evaluating the antimicrobial efficacy of these 3 oral antiseptics, unlike those evaluating the antimicrobial efficacy of chlorhexidine. In addition, concerns have been raised about the emergence of microorganisms resistant to chlorhexidine, which appears not to be a clinical problem yet. The aim of the present in vitro study was to compare the antimicrobial activity of 3 commercially available, novel oral antiseptics (containing octenidine, polyhexamethylene biguanide, or Citrox, respectively) with that of chlorhexidine.

**METHODS**

**Microorganisms and Growth Conditions**

The DSM 20068 strain of *Streptococcus sanguinis* and the DSM 20523 strain of *Streptococcus mutans* were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, and the ATCC 90028 strain of *Candida albicans* and the ATCC 10953 strain of *Fusobacterium nucleatum* were obtained from the American Type Culture Collection. The strains were grown on Columbia blood agar plates (BBL; Becton Dickinson) supplemented with 50 mL/L of human blood, 0.5 mg/L of menadione, and 5 mg/L of hemin at 37°C. *C. albicans* was incubated under aerobic conditions for 24 hours; *S. mutans* was incubated in air with 10% CO₂, 10% H₂, and 80% N₂ for 24 hours; and *F. nucleatum* was incubated under anaerobic conditions for 48 hours. These conditions were used throughout our study.

**Preparation of Test Solutions**

The following 3 commercially available oral antiseptics were compared: Octenidol (Schülke & Mayr AG), which contains octenidine as an antimicrobial agent; ProntOral (B. Braun Medical), which contains polyhexamethylene biguanide as an antimicrobial agent; and OralClens (Oraldent), which contains Citrox as an antimicrobial agent. The exact formulas for these mouth rinses are not in the public domain because of patent-related restrictions. Chlorhexidine digluconate solution in a frequently used and recommended concentration (0.2%) (Sigma) served as a comparator. These commercially available mouth rinses were applied in their recommended concentration and also at serial dilutions down to 10⁻³ to test lower concentrations as they may occur during application.

**Broth Dilution Assay**

The test strains were suspended in 0.9% NaCl, harvested by centrifugation at 10,000 rpm (radius, 85 mm) for 5 minutes, and resuspended in 0.9% NaCl. The microbial suspensions were adjusted to a final cell density of approximately 6 × 10⁶ CFU/mL, controlled by plating appropriate dilutions of the suspensions onto the Columbia blood agar plates. An aliquot of 100 μL of a microbial suspension was added to 900 μL of a test solution and mixed thoroughly. After incubation at room temperature for 1 minute, the suspensions were serially diluted in 0.9% NaCl, and appropriate dilutions were plated onto Columbia blood agar media. The number of colony-forming units per milliliter was determined after strain-specific incubation, and the mean log₁₀ CFU reduction factor was calculated. The antimicrobial activity of each antiseptic was assessed by counting the difference in bacterial densities (ie, the log₁₀ number of colony-forming units of bacteria) before and after the disinfection process. All experiments were performed 3 times.

**Agar Diffusion Assay**

A modified agar diffusion assay was used. In brief, 20 μL of undiluted or diluted mouth rinse was placed onto a blank, nonimpregnated filter-paper disk (bioMérieux; diameter, 6 mm). Sterilized 0.9% NaCl served as a negative control. Suspensions equal to the 0.5 McFarland standard for each bacterial strain and suspensions equal to 2–4 McFarland for *C. albicans* were prepared in broth medium: Sabouraud dextrose broth (Becton Dickinson) for *C. albicans*, Schaedler broth (Becton Dickinson) for *S. mutans* and *S. sanguinis*, and thio-glycolate broth (Becton Dickinson) for *F. nucleatum*. After inoculating the suspensions evenly with a cotton swab onto Columbia blood agar plates, the disks were placed and gently
pressed on the plates with sterile forceps. After strain-specific incubation, all plates were photographed, and the inhibition zones were measured. All experiments were performed 3 times.

Statistical Analyses

All analyses were done by use of the statistical package R, version 2.8 (The R Foundation for Statistical Computing). Prior to the statistical analyses, all colony-forming units per milliliter were log-transformed. Statistical comparisons were made for the results obtained for the undiluted mouth rinses. For each strain, linear regression models were performed, with “diameter” and “count reduction” as dependent factors and “treatment” as the independent factor. Pairwise differences in the mean values were calculated between each treatment for each strain independently. Ninety-five percent confidence intervals and 2-sided P values were calculated. P values of less than 0.05 were considered to be statistically significant. P values were adjusted for multiple comparisons according to the Tukey method. Box plots were prepared that show the dependency of the count reduction and the inhibition zone on the treatment and concentration of the test solutions. The efficacy of a mouth rinse was calculated as a log$_{10}$ reduction factor after the mouth rinse was exposed for 1 minute in a broth medium.

RESULTS

In general, the antimicrobial activity of the mouth rinses containing octenidine and polyhexamethylene biguanide was comparable to that of chlorhexidine. In contrast, the mouth rinse containing Citroxx showed remarkably weaker antimicrobial efficacy. Dose-dependent killing was observed in all experiments, whereas the cell counts of the negative control (ie, 0.9% NaCl) remained unaffected.

S. sanguinis

In both the broth dilution assay (Figure 1A) and the agar diffusion assay (Figure 1B), similar antimicrobial activity against *S. sanguinis* was demonstrated by Octenidol and ProntOral. The broth dilution assay revealed that Octenidol and ProntOral were active against *S. sanguinis* even at very low concentrations, whereas chlorhexidine demonstrated only a moderate effect. In contrast, the undiluted chlorhexidine was the most effective solution in the agar diffusion assay. In the agar disk assay, the disks treated with undiluted OralClen showed an inhibition zone comparable to the disks treated with undiluted Octenidol or undiluted ProntOral. In the broth dilution assay, no antimicrobial efficacy was evident for OralClen (mean reduction factor, 0.26 CFU/mL). The mean reduction factor was 7.2 CFU/mL for undiluted Octenidol, 7.2 CFU/mL for undiluted ProntOral, and 1.03 CFU/mL for undiluted chlorhexidine. In the broth dilution assay, there were statistically significantly lower microbial counts for chlorhexidine (P < .001) and OralClen (P < .001), compared with ProntOral and Octenidol. The diameters of the inhibition zones were statistically significant for Octenidol, compared with chlorhexidine (P < .001) or ProntOral (P < .001).

**Figure 1.** Box plot summarizing the log$_{10}$ reduction of total microbial counts (ie, the reduction factor, in units of CFU/mL) and the diameters of the inhibition zones obtained when testing different concentrations of the mouth rinses against *Streptococcus sanguinis*. The point represents the mean value. The top and bottom borders of the box represent the 75th and 25th percentiles, respectively. The whiskers above and/or below the box represent the 90th and 10th percentiles. CHX, chlorhexidine.
Figure 2. Box plot summarizing the log_{10} reduction of total microbial counts (ie, the reduction factor, in units of CFU/mL) and the diameters of the inhibition zones obtained when testing different concentrations of the mouth rinses against Streptococcus mutans. The point represents the mean value. The top and bottom borders of the box represent the 75th and 25th percentiles, respectively. The whiskers above and/or below the box represent the 90th and 10th percentiles. CHX, chlorhexidine.

Figure 3. Box plot summarizing the log_{10} reduction of total microbial counts (ie, the reduction factor, in units of CFU/mL) and the diameters of the inhibition zones obtained when testing different concentrations of the mouth rinses against Fusobacterium nucleatum. The point represents the mean value. The top and bottom borders of the box represent the 75th and 25th percentiles, respectively. The whiskers above and/or below the box represent the 90th and 10th percentiles. CHX, chlorhexidine.

S. mutans

S. mutans was more susceptible to chlorhexidine, Octenidol, and ProntOral than was S. sanguinis. Concentrations of Octenidol and ProntOral as low as 1% were able to kill this microorganism in the broth dilution assay (Figure 2A). As with S. sanguinis, no reduction in the number of colony-forming units per milliliter could be detected when S. mutans was treated with OralClens, whereas modest inhibitory activity was shown in the agar diffusion assay (Figure 2B). The

0.03; for OralClens, compared with ProntOral (P < 0.03); and for chlorhexidine, compared with OralClens (P < 0.001).
Mean reduction factor was 8.24 CFU/mL for undiluted Oc-tenidol, 8.24 CFU/mL for undiluted ProntOral, 0.22 CFU/ mL for undiluted OralClens, and 8.24 CFU/mL for undilut-ed chlorhexidine. A statistically significant difference in the reduction in microbial counts with chlorhexidine, Octenidol, and ProntOral, compared with OralClens (P < .001), was found using the highest concentration of the mouth rinses in the broth dilution assay. In the agar diffusion assay, the inhibition zone for chlorhexidine was statistically significantly different from the inhibition zones for OralClens (P < .001), Octenidol (P < .001), and ProntOral (P < .001). The inhibition zones for Octenidol (P = .02) and ProntOral (P < .001) were statistically significantly different from the inhibition zone for OralClens.

**F. nucleatum**

Both the broth dilution assay (Figure 3A) and the agar diffu-sion assay (Figure 3B) showed that Octenidol, ProntOral, and chlorhexidine are effective against *F. nucleatum*. ProntOral demonstrated remarkable antimicrobial efficacy even at the lowest concentration in the broth dilution assay. Chlorhexidine and Octenidol were also potent against *F. nucleatum*, whereas OralClens showed only a minor killing effect in the broth dilution assay and failed to inhibit growth in the agar diffusion assay. The mean reduction factor was 7.1 CFU/mL for undiluted Octenidol, 7.1 CFU/mL for undiluted ProntOral, 1.36 CFU/mL for undiluted OralClens, and 7.1 CFU/mL for undiluted chlorhexidine. Compared with OralClens, when the highest concentrations of chlorhex-

idine (P < .001), Octenidol (P < .001), and ProntOral (P < .001) were used in the broth dilution assay, the reductions in microbial counts were statistically significant. The diameters of the inhibition zones on the disks treated with chlorhexi-dine, OralClens, Octenidol, and ProntOral differed statistically significantly in the agar diffusion assay, depending on whether the highest concentration of the mouth rinses or the chlorhexidine control solution was used (OralClens vs chlorhexidine, ProntOral, and Octenidol $[P < .001]$; Octenidol vs ProntOral $[P = .02]$; chlorhexidine vs Octenidol and Pront-Oral $[P < .001]$).

**C. albicans**

ProntOral, Octenidol, and chlorhexidine were effective even at low concentrations in the broth dilution assay (Figure 4A) and in the agar diffusion assay (Figure 4B). Only the undiluted OralClens showed inhibition in the agar diffusion assay. The mean reduction factor was 7.54 CFU/mL for undiluted Octenidol, 7.54 CFU/mL for undiluted ProntOral, 0.35 CFU/ mL for undiluted OralClens, and 7.54 CFU/mL for undiluted chlorhexidine. Statistically significant differences in the re-

duction in microbial counts with chlorhexidine, OralClens, and ProntOral, compared with OralClens (P < .001), were found using the highest concentrations of the solutions in the broth dilution assay. In the agar diffusion assay, statisti-cally significant differences were found for disks treated with ProntOral, compared with OralClens (P = .01), Octenidol (P = .01), and chlorhexidine (P = .01).
DISCUSSION
Our in vitro study clearly demonstrated that the efficacy of the mouth rinses containing octenidine or polyhexamethylene biguanide (but not Citroxx) was similar to the efficacy of chlorhexidine. Octenidine and polyhexamethylene biguanide remained very active even when highly diluted. In contrast, chlorhexidine lost its antimicrobial efficacy when diluted to less than 10% of its original concentration. Therefore, it is conceivable that such mouth rinses are at least as effective as chlorhexidine, on basis of these in vitro experiments. In fact, these products may be even more effective than chlorhexidine, because many-fold dilutions are to be expected in mouth rinses that are used by the general public. Similar to chlorhexidine, octenidine and polyhexamethylene biguanide have prolonged effects after application.

The oral cavity is the natural habitat of a wide variety of microorganisms.20 Some of them may be beneficial to the host, while others are considered to play a role in the pathogenesis of certain oral or other infectious diseases, including ventilator-associated pneumonia.20-23 Ideally, microorganisms beneficial to the host should not be affected by an antiseptic. The selection of the species in the present study represents some of the microorganisms with documented key characteristics in the oral habitat or for the pathogenesis of pulmonary infections. S. sanguinis is commonly found in the oral cavity, is not considered pathogenic, and may be beneficial to the host. This bacterium is thought to demonstrate a competitive inhibition of S. mutans,24,25 whereas S. mutans is closely related to the pathogenesis of caries.22 F. nucleatum, a gram-negative anaerobic rod, is an important link between the primary and secondary colonizers in the dental plaque and is detected in chronic periodontitis lesions and frequently recovered from patients with necrotizing-ulcerative gingivitis and/or periodontitis.23,26 In contrast to the treatment of chronic periodontitis, emergency treatment of necrotizing-ulcerative gingivitis and/or periodontitis consists of mechanical debridement with adjunctive use of antiseptics.

F. nucleatum may also cause ventilator-associated pneumonia or other severe infections.2,27 The yeast C. albicans lives in a state of commensalism in the oral cavity but is also an important opportunistic pathogen that can cause infections varying from harmless newborn thrush to severe deep mucocutaneous candidiasis or even sepsis in patients with a local or systemic compromised immune response or in patients who take antibiotics.28-30

Our investigation has revealed that the killing efficacy of chlorhexidine against S. sanguinis was weaker than that of mouth rinses containing octenidine or polyhexamethylene biguanide (Figure 1). This finding is in accordance with that of Decker et al.31 In ascending order, the following mouth rinses were found to be the most effective against S. mutans: those containing chlorhexidine, those containing polyhexamethylene biguanide, and those containing octenidine. This confirms the recent findings of the comparative antimicrobial efficacy of octenidine and chlorhexidine in saliva (Figure 2).32 Thus, octenidine killed streptococci most efficiently, whereas polyhexamethylene biguanide demonstrated superior antifungal activity and control of anaerobic gram-negative F. nucleatum (Figures 3 and 4). The mouth rinse containing Citroxx, which consists of natural antimicrobial agents (including bioflavinoids and fruit acids), demonstrated significantly weaker antimicrobial efficacy than did the other agents tested. Therefore, its usefulness in antimicrobial mouth rinses is questionable.

Clinical studies have shown that mouth rinses containing octenidine are effective in the control of dental plaque.33,34 The efficacy of polyhexamethylene biguanide with regard to bacterial counts, dental plaque, and the 4-day regrowth of plaque has also been investigated.35,36 This antimicrobial agent seems to inhibit plaque recolonization and to reduce oral bacterial counts. The present in vitro data are in accordance with previous clinical reports. However, the nature of the present study is not just confirmatory, because the relative efficacy of these novel and commercially available mouth rinses has hitherto not been tested. Although the antimicrobial agents were given, the exact formulas of the mouth rinses were not available. Thus, the mode of action of the Vagents given may have been affected by interactions with other ingredients.

No neutralizing agent was used in the current investigation, which might be a critical issue (because of the possible prolonged effect of the tested agent) that could probably lead to higher observed reduction factors. However, all of the compounds were tested using the exact same assays and test conditions, indicating valid results. In practice, mouth rinses are used for 30 seconds to 1 minute and then are spit out. Most chemical compounds (including chlorhexidine, octenidine, and polyhexamethylene biguanide) are very tenacious, which means that they bind well to tissue (resulting in a depot effect). Within the limitations of our in vitro study, we found the mouth rinses containing octenidine or polyhexamethylene biguanide to be potent alternative oral antiseptics to chlorhexidine.

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