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ORIGINAL ARTICLE

Antibacterial and antiplaque efficacy of a commercially available octenidine-containing mouthrinse

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Abstract
Objectives The purpose of this clinical study was to determine the antibacterial and antiplaque efficacy of a recently introduced octenidine-containing mouthrinse (Octenidol®) in comparison with established antiseptic mouthrinses.
Materials and methods In a 4-day plaque-regrowth study employing a four-replicate cross-over design, a 0.1 % octenidine mouthrinse (Octenidol®/OCT-MR) was compared with a 0.12 % chlorhexidine mouthrinse (Paroex®/CHX-MR), an essential oil mouthrinse (Listerine®/EO-MR), and a placebo mouthrinse/P-MR. Plaque regrowth was assessed with a modified Quigley-Hein plaque index. The antibacterial effect was assessed by taking bacterial counts from the tooth surface and oral mucosa after professional tooth cleaning and after first rinsing with the allocated mouthrinse on days 1 and 5. Sixteen volunteers suspended tooth cleaning and rinsed twice daily with the allocated mouthrinse for 4 days.
Results All tested antiseptic mouthrinses were significantly more effective than the placebo mouthrinse in inhibiting plaque, but no significant differences were observed between OCT-MR and CHX-MR, OCT-MR and EO-MR, and CHX-MR and EO-MR. After 4 days, comparable bacterial count levels were found on both the tooth surface and mucosa applying OCT-MR and CHX-MR, which were significantly lower than that of EO-MR and P-MR.
Conclusion Octenidol® and Paroex® showed comparable antibacterial and antiplaque efficacy in the human oral cavity.
Clinical Relevance The recently introduced octenidine-containing mouthrinse Octenidol® may become a suitable alternative to 0.12 % chlorhexidine-containing mouthrinses such as Paroex®.

Keywords Mouthrinses · Antiseptic · Octenidine · Chlorhexidine · Essential oil · Dental plaque

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Introduction

Due to the microbial nature of dental plaque as the initial factor of oral diseases such as dental caries, gingivitis, and periodontitis, the use of antiseptic mouthrinses as an adjuvant to daily mechanical tooth cleaning is recommended [1, 2], especially for high-risk groups, e.g., patients undergoing fixed orthodontic therapy or those with mental and/or physical disabilities [3, 4].

Chlorhexidine digluconate (CHX) has been used for over 40 years and is still considered the most effective oral hygiene agent in plaque inhibition. To date, no microbial resistance or a shift in the oral microflora has been observed in conjunction with its use [5, 6]. However, CHX has a limited use period because of its known side effects, such as discoloration [7, 8], mucosal irritation, and taste alteration [9]. Further, CHX can cause allergic reactions and in very rare cases anaphylaxis [10].

Therefore, other antiseptic agents are being sought which are comparable to the common CHX mouthrinses in terms of

its efficacy to reduce oral bacteria counts and inhibit plaque growth.

The antiseptic agent octenidine dihydrochloride (OCT) (Fig. 1) was developed by Sterling Winthrop (WIN 41464-2) in the 1980s [11] as an alternative to the antiplaque agent CHX and successfully tested in vitro and in vivo [12–16]. OCT has a broad spectrum of activity with similar effectiveness against not only Gram-negative and Gram-positive bacteria [17, 18] but also fungi and yeasts [19, 20]. Moreover, it has also been shown that OCT may effectively interfere with co-aggregation of dental plaque microbial colonizers without disturbing the normal, healthy oral flora [21].

In a standardized comparison of antimicrobial activity of the antiseptic agents triclosan, polyvinylpyrrolidone-iodine, OCT, polyhexamethylene biguanide, and CHX using the minimal effective concentration and minimum bactericidal concentration, OCT was the most effective active agent of all tested antimicrobial substances [22]. In other studies, the antibacterial and antiplaque efficacy of OCT was equal to or even greater than that of CHX [23, 24].

In spite of these verified effects, OCT has not been applied as a mouthrinse or an antiplaque agent in the past due to its very bitter taste.

The 0.1 % OCT-containing mouthwash solution Octenidol®, which contains supplements and flavorings to mask the unpleasant taste of OCT, was recently introduced on the market [25], but no clinical studies on its efficacy exist to date.

The purpose of the present study was to compare the effect of the 0.1 % octenidine-containing mouthrinse Octenidol®/OCT-MR on bacteria and plaque formation with the 0.12 % chlorhexidine-containing mouthrinse Paroex®/CHX-MR as positive control, the commercially available essential oil-containing mouthrinse Listerine®/EO-MR as benchmark control, and a placebo mouthrinse/P-MR as negative control.

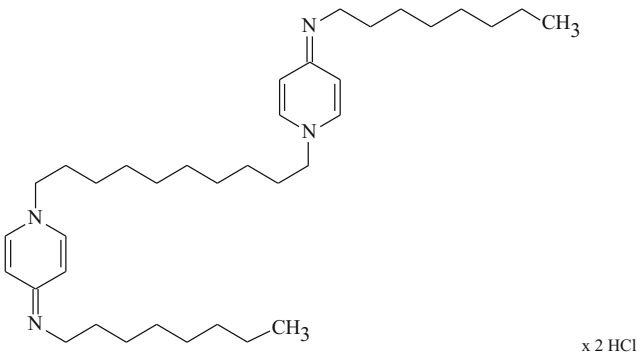

Fig. 1 Structural formula of octenidine dihydrochloride {1,1′-decamethylene-bis[1,4-dihydro-4-(octylimino)-pyridin]dihydrochloride, or N,N′-[1,10-decandiyldi-1(4H)pyridinyl-4-ylidene]bis(1-octanamine)dihydrochloride}

Materials and methods

Study design and subjects

The study employed a double-blind, placebo-controlled, randomized, four-replicate cross-over design, described by Addy et al., in which each subject served as its own control [26]. Sixteen healthy volunteer students of the Dental School of the University Medicine Greifswald (4 males and 12 females, mean age 25 years) were selected who had a good standard of oral hygiene with an approximal plaque index of <25 %, good gingival health with a sulcus bleeding index of <10 %, and at least 25 scorable teeth [27, 28]. Exclusion criteria were wearing dentures, concurrent participation in another clinical trial, pregnancy, lack of compliance, taking antibiotics, alcohol or drug addicts, and known allergy or sensitization during the application to an ingredient of the test or control mouthrinses. The study was approved by the Ethics Committee of the University of Greifswald (BB 99/12). Screening and selection of subjects were exclusively performed by the investigator (CB) who also took all clinical parameters in all four test cycles.

Every subject rinsed with all four tested mouthrinses over the whole study period. Before the first of four test cycles began, the subjects were randomly assigned a number, which determined the order of application of the following mouthrinses:

- OCT-MR: Octenidol® (Schülke Plus GmbH, Germany), containing 0.1 % octenidine dihydrochloride in an aqueous solution additionally supplemented with PEG-40 hydrogenated castor oil, glycerol, artificial flavoring, sodium gluconate, sucralose, citric acid, and butylated hydroxytoluene.
- CHX-MR: Paroex® (Sunstar Suisse S.A., Switzerland), containing 0.12 % chlorhexidine digluconate in an aqueous solution additionally supplemented with propylene glycol, glycerol, PEG-40 hydrogenated castor oil, artificial flavoring, potassium acesulfame, CI 14720, methylparaben, and limonene.
- EO-MR: Listerine® cool mint™ (Johnson & Johnson GmbH, Germany), containing the essential oils (EO) menthol (0.042 %), thymol (0.064 %) and eucalyptol (0.092 %), methyl salicylate (0.06 %), and 21.6 % (*v*/*v*) ethanol to dissolve the active ingredients.
- P-MR: Placebo solution, containing 0.5 % Tween® 20, 0.05 % (*v*/*v*) peppermint oil (Minthea peperita, Lavita) and 0.005 % food coloring solution (green). In a separate experiment, no antimicrobial effect was detected on oral bacteria using quantitative suspension tests in accordance with EN 1040 [29].

All four rinses were filled in opaque bottles of identical appearance and coded. The codes were unknown to the subjects and clinical investigator. Compliance of volunteers was assessed by measuring the residual volume of mouthrinse in the bottles.

Reproducibility of clinical examinations

The clinical investigator (CB) was given intensive clinical training, during which this investigator was calibrated against a very experienced investigator (AW) to achieve high reproducibility of the plaque index scores. A sufficient inter- and intra-reliability of $k \geq 0.79$ was achieved for the plaque index.

Clinical trial

The sequence of the clinical study and time points of sampling are outlined in Fig. 2.

Bacterial count measurements

The antibacterial efficacy of the mouthrinses was assessed by determining bacterial counts on both the tooth (no. 26) and mucosa immediately after professional tooth cleaning and after mouthrinsing on day 1 and on day 5 after the last rinse on the evening before. The smears on the tooth surfaces were taken using a sterile cotton swab (REF 1020003, Heinz Herens Medizinalbedarf GmbH, Germany) and that of the buccal mucosa with a sterile calcium alginate swab (14-959-81, Fisherbrand, USA).

The samples were aseptically immersed in 5 ml Lipofundin® MCT 20 % (B. Braun, Melsungen, Germany) containing 1.2 % (*w*/*v*) egg yolk phosphatidylcholine to inactivate antiseptic agents; this was validated in separate experiments as recommended [30]. The swabs were stored at 4 °C until analysis within 8–10 h after taking smears. For analysis, the samples were vigorously vortexed for 1 min. Thereafter, three serial dilutions (1:10) were prepared in trypticase soy broth, and 0.1 ml of each dilution was plated in triplicate on trypticase soy agar (Oxoid, Wesel, Germany). The colony forming units (CFU) of microorganisms were counted after 48 h of incubation at 37 °C. Results were calculated as CFU/sample.

Plaque regrowth study

The antiplaque efficacy of the tested mouthrinses was assessed by measuring the plaque regrowth after 4 days [26].

On the first day of each study period, an intraoral examination of the teeth and soft tissue was followed by professional tooth cleaning and polishing of the teeth to remove tartar, plaque, soft deposits, and all external tooth stains.

After rinsing for 30 s with tap water, the first smears from the buccal surface of tooth 26 (maxillary first molar, left) and the buccal mucosa on the opposite side were taken. Subjects then rinsed for 1 min with 20 ml of their allocated mouthrinse.

A resampling of smears from the tooth surface and buccal mucosa was performed. The subjects rinsed again in the evening for 1 min with 20 ml of their assigned mouthrinse on day 1. On the following 3 days, the volunteers suspended their normal oral hygiene habits and rinsed twice a day (after breakfast and in the evening) for 1 min with 20 ml of their allocated mouthrinse. The last rinse was performed in the evening of day 4. The clinical evaluation took place on day 5 by taking smears from the mucosa and the tooth surface. Then, the accumulated plaque was disclosed using a disclosing solution (MIRA-2-TON®, Hager & Werken GmbH, Germany) and scored using the Turesky et al. [31] modification of the Quigley-Hein plaque index (QHI) [32]. The scores were taken at six surfaces per tooth: mesio-, mid-, disto-buccal, mesio-, mid-, and disto-lingual tooth surfaces. After completing the assessments, each volunteer received professional full-mouth scaling and polishing.

Each test cycle was followed by a 10-day wash-out period, in which the subjects resumed their normal oral hygiene habits.

Statistical methods

The plaque index scores were normally distributed and analyzed without transformation.

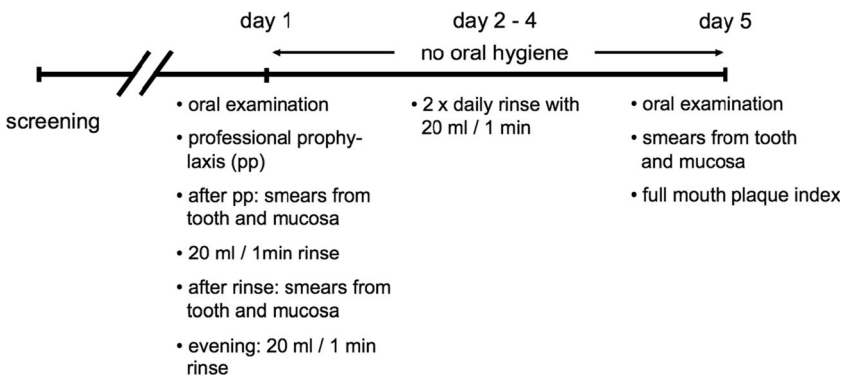

Fig. 2 Outline of the clinical test cycle, conducted four times

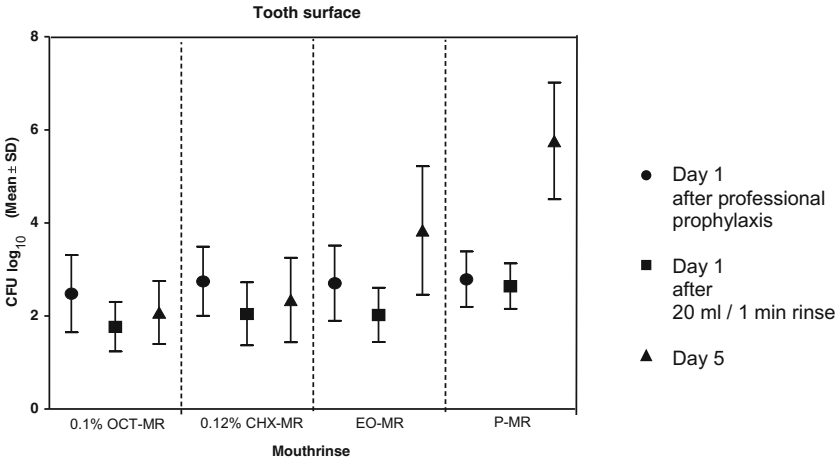

Fig. 3 Bacterial counts (colony forming units per sample, log transformed) on the tooth surface: means, estimated 95 % confidence intervals, and results of ANOVA (adjustment multiple comparison) for four treatments ($N = 16$)

Analysis of variance (ANOVA) was used to determine the effects of treatment, subject, and period. Differences between pairs of treatments were determined by 95 % confidence intervals with Tukey HSD adjustment for multiple comparisons (significance level $\alpha = 0.05$).

Bacterial counts (CFU/sample) were positively skewed and required log transformation to fit a normal Gaussian model. ANOVA followed by Bonferroni HSD adjustment for multiple comparisons with a significance level ($\alpha = 0.05$) was used to measure the differences between pairs of treatments.

Results

Bacterial counts

On day 1 of each clinical test cycle, professional tooth cleaning resulted in comparable bacterial counts on tooth surfaces (Fig. 3) and oral mucosa (Fig. 4) in each test group. The reduction of bacterial counts was higher after rinsing with mouthrinses compared with the results of tooth cleaning alone. On the tooth surfaces, the reduction of bacterial counts differed significantly only from placebo after rinsing with 0.1 % OCT-MR but not after 0.12 % CHX-MR and EO-MR (Fig. 3 and Table 1). On the other hand, mouthrinses with 0.1 % OCT-MR, 0.12 % CHX-MR, and EO-MR were significantly more effective than placebo in reducing the bacterial counts on the oral mucosa (Fig. 4 and Table 2).

On day 5 (after 4 days of suspending oral hygiene habits), there was a recolonization of bacteria on both the tooth surface (Fig. 3) and oral mucosa (Fig. 4). The increase of bacterial counts was more evident in the placebo group on both surfaces in the oral cavity (Figs. 3 and 4). The bacterial counts on the tooth surface were significantly lower after rinsing with 0.1 % OCT-MR and 0.12 % CHX-MR in comparison to EO-MR and P-MR (Fig. 3). The inhibition of the bacterial growth was more effective after using 0.1 % OCT-MR than 0.12 % CHX-MR, but this difference was not significant (Table 1).

On the oral mucosa, a reduction of bacterial counts was also detected with all three antiseptic mouthrinses. However, only 0.1 % OCT-MR and 0.12 % CHX-MR showed

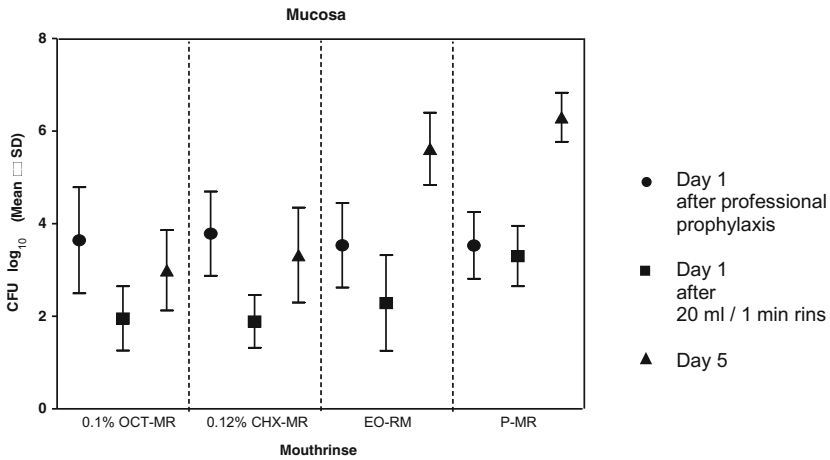

Fig. 4 Bacterial counts (colony forming units per sample, log transformed) on the oral mucosa: means, estimated 95 % confidence interval, and results of ANOVA (adjustment for multiple comparison) for four treatments ($N = 16$)

Table 1 Results (*p* values) based on the paired comparison of mean bacterial counts of the treatments on the tooth surface, resulting from ANOVA

	Day 5[+]			
	0.1% OCT-MR	0.12% CHX-MR	EO-MR	P-MR
Day 1[++]				
0.1% OCT-MR		1.000	**0.000**	**0.000**
0.12% CHX-MR	0.781		**0.004**	**0.000**
EO-MR	0.866	1.000		**0.000**
P-MR	**0.003**	0.190	0.167	

Bacterial counts after first rinse on day 1 (lower left panel—below the diagonal) and on day 5 (upper right panel—above the diagonal). Bonferroni adjustment as multiple-comparison technique for ANOVA was used (significance level $\alpha = 0.05$). Significant *p* values are bolded.

[+] Adjusted R2 = 0.658; [++] adjusted R2 = 0.248

significantly lower bacterial counts than P-MR. The antibacterial efficacy of 0.1 % OCT-MR and 0.12 % CHX-MR did not differ significantly from each other (Table 2).

Plaque regrowth study

The results of plaque regrowth (mean QHI) for each tested mouthrinse after 4 days of usage are shown in Fig. 5. Compared to the P-MR (2.35), 0.1 % OCT-MR (1.23), 0.12 % CHX (0.99), and EO-MR (1.40) were significantly more effective in plaque inhibition. The differences among the three antiseptic mouthrinses were not significant (Fig. 5).

Discussion

The results of this study revealed comparable antimicrobial and antiplaque efficacy of the 0.1 % OCT-containing commercially available mouthrinse Octenidol® and the 0.12 % CHX containing mouthrinse Paroex®.

The plaque regrowth design of Addy et al. [26] applied here is considered a recommended method to evaluate the efficacy of an antimicrobial and antiplaque agent [33, 34].

A special feature of superficially adhering antiseptic agents is the pronounced residual antimicrobial effect defined as substantivity, which is the most important factor for being an effective antimicrobial agent, because it is not only important to have an immediate high antimicrobial effect but also to continue its therapeutic activity for a prolonged period of time [35–38].

The determination of oral bacterial counts is considered to be predictive of the antibacterial agent's substantivity [39–41] and its potential antiplaque activity [42]. The good antiplaque efficacy of CHX depends primarily on the persistence of its antibacterial activity on oral mucosa and tooth surfaces, which can be measured by bacterial count reduction tests up to 7 h [38, 43].

In order to compare the substantivity of Octenidol® with that of Paroex® and Listerine®, we measured bacterial count reductions on the surfaces where the bacteria actually grow. However, the best sampling time point for the bacterial cultures is not easy to determine. The professional tooth cleaning on day 1 was conducted until a QHI of 0 was obtained. In contrast to mucosal smears, the bacteria of the tooth smears—taken directly after mechanical tooth cleaning—did not originate only from the sample surface: microflora from saliva and mucosa contaminated the tooth surface during the rinsing with water. However, the resulting bacterial counts on both mucosa and tooth of the different test groups showed comparable statistically similar starting conditions without significant differences (Figs. 3 and 4).

The subjects were asked to rinse the oral cavity with their allocated mouthrinses until the evening of day 4. Thus, the sampling on day 5 took place 12–16 h after the last rinsing, which showed that the substantivity of the active agents CHX of Paroex® and OCT of Octenidol® was still evident after the last rinse, which was not the case with placebo and Listerine®. The difference of a maximum of 4 h should have no relevant influence on the results and can be neglected, because each

Table 2 Results (*p* values) based on the paired comparison of mean bacterial counts of the treatments on the mucosa, resulting from ANOVA

	Day 5[+]			
	0.1% OCT-MR	0.12% CHX-MR	EO-MR	P-MR
Day 1[++]				
0.1% OCT-MR		1.000	**0.000**	**0.000**
0.12% CHX-MR	1.000		**0.000**	**0.000**
EO-MR	1.000	1.000		0.108
P-MR	**0.000**	**0.001**	**0.007**	

Bacterial counts after first rinse on day 1 (lower left panel—below the diagonal) and on day 5 (upper right panel—above the diagonal). Bonferroni adjustment as multiple-comparison technique for ANOVA was used (significance level $\alpha = 0.05$). Significant *p* values are bolded.

[+] Adjusted R2 = 0.764; [++] adjusted R2 = 0.372

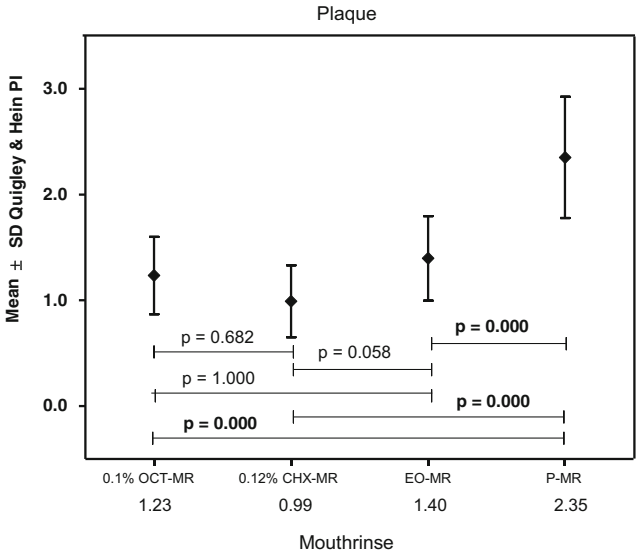

Fig. 5 Four-day plaque regrowth study: means, estimated 95 % confidence intervals, and results of ANOVA for four treatments ($N = 16$)

volunteer was randomly assigned to a measurement time point in each study period.

The official recommended use of 0.12 % CHX solution Paroex® is twice 10 ml per day. In the present study, the amount was doubled to 20 ml to get the minimum concentration of the gold standard (40 mg CHX per day). However, in the interpretation of the findings, it should be borne in mind that there are no equivalence studies of Paroex® to the gold standard (7) and additional ingredients of commercially available mouthrinses may reduce the antimicrobial efficacy of their active agent [34, 44–46].

The antibacterial efficacy of Listerine® directly after mouthrinsing on day 1 was comparable to that of Paroex® and Octenidol®. However, Paroex® and Octenidol® had better antibacterial efficacy than did Listerine® after 4 days, which indicates that Listerine® has high antibacterial efficacy but modest substantivity. This was also supported by the tendency of the mean QHI scores: Paroex® (0.99), Octenidol® (1.23), Listerine® (1.40), and placebo (2.35) (Fig. 5). The presented results support the applicability of OCT as an antimicrobial and plaque-controlling agent as demonstrated in earlier in vivo studies [13, 15, 16, 47]. The results are also in line with further other studies [12, 24, 48–50]. For instance, the aqueous mucous membrane antiseptic Octenisept® containing 0.1 % OCT was found to be more effective than 0.2 % CHX in substantially reducing total salivary and total cariogenic bacterial counts. Additionally, during 4 days of usage, 0.1 % OCT was the most effective mouth rinse in reduction of total salivary and cariogenic bacterial counts compared with that of 0.2 % CHX and 7.5 % polyvinylpyrrolidone-iodine complex [24]. Moreover, 0.5 % OCT was also as effective as 0.5 % CHX in decreasing plaque scores in rats [48]. In another study, OCT in a concentration of 1 % reduced plaque scores significantly better than 1 % CHX after 7 days of application in monkeys [12].

Among other antiseptics, Octenidol® was also tested in vitro by Rohrer et al. [49]. Octenidol® demonstrated antimicrobial activity comparable to that of 0.2 % CHX after testing the four common oral microorganisms (*Streptococcus sanguinis*, *Streptococcus mutans*, *Candida albicans*, and *Fusobacterium nucleatum*) under standard conditions. The authors concluded that OCT and CHX are very tenacious, which means that they bind well to tissue, resulting in a depot effect, and considered OCT as a potent alternative to CHX-containing preparations [49]. This is supported by another study in which the challenge of protein, blood, or mucin did not markedly alter the antimicrobial efficacy of CHX and OCT [50].

Conclusion

According to the presented results, the recently introduced 0.1 % OCT-containing mouthrinse Octenidol® revealed antibacterial and antiplaque efficacy comparable to that of the 0.12 % CHX-containing mouthrinse Paroex® in the human oral cavity. Thus, Octenidol® may become an alternative to commercially available 0.12 % CHX-containing mouthrinses such as Paroex®. However, further clinical studies are needed to evaluate its safety and efficacy in long-term use.

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Compliance with ethical standards The study was approved by the Ethics Committee of the University of Greifswald (BB 99/12).

Conflict of interest The authors declare that they have no competing interests.

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