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Short-term relative antibacterial effect of octenidine dihydrochloride on the oral microflora in orthodontically treated patients

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KEYWORDS
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Summary
Objective: To evaluate the efficacy of common antiseptic mouth rinses and octenidine dihydrochloride (OCT).
Methods: The antibacterial activities of antiseptics against total and cariogenic bacteria (*Streptococcus mutans* and *Lactobacillus* species) in saliva were studied in vitro and in vivo. After unstimulated saliva was collected, one of the mouth rinse solutions was applied for 30 seconds. Saliva samples were collected 15, 30, 60, and 120 min later and evaluated for their bacterial count.
Results: OCT had a significantly greater inhibitory effect on the studied bacteria than 0.2% chlorhexidine gluconate (CHX) and 7.5% polyvinylpyrrolidone—iodine complex (PVP—I) from 15 min to 120 min following the application ($p < 0.01$). The antiseptic efficacy of 0.2% CHX on total bacteria and *Lactobacillus* species was very similar to the efficacy observed with 7.5% PVP—I mouth solution from 15 min up to 120 min. *Streptococcus mutans* was completely inhibited by 0.15 mg/ml PVP—I, 0.5 mg/ml CHX, and 0.1 mg/ml OCT concentrations, while *Streptococcus salivarius* was inhibited by 0.15 mg/ml PVP—I, 2 mg/ml CHX, and 0.8 mg/ml OCT concentrations. *Lactococcus lactis* subspecies *lactis* was inhibited with 0.00313 mg/ml OCT, 30 mg/ml PVP—I, and 0.0063 mg/ml CHX concentrations.
Conclusions: OCT compared favorably with CHX and PVP—I in its antibacterial effects, both in vitro and in vivo ($p < 0.01$).
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Introduction

Fixed orthodontic appliances are considered to be a clinical risk factor in terms of enamel integrity because of plaque accumulation around the bracket bases.[1] The placement of orthodontic appliances generally hinders good oral hygiene, and the appliance components can cause alterations in the oral microflora by reducing pH and increasing retention areas for microorganisms.[2,3] Increased levels of *Streptococcus mutans* and *Lactobacillus* species have been detected in the oral cavity and saliva after bonding orthodontic attachments.[2,4,5] These microorganisms have been identified as the main pathogens in dental caries, and their presence increases the risk for decalcification.[5]

Patient success in maintaining proper hygiene appears to be limited by the patient's inability to reach certain plaque-infected sites because of the appliances used during orthodontic treatment. Hence, there currently exists a great need for the development of antibacterial chemotherapeutic agents to assist in the control of dental plaque-associated infections in humans.[6,7] Some antibacterial agents are able to permeate the plaque matrix and rapidly kill pathogenic bacteria, thereby helping to reduce the destructive effect of microorganisms that create an acidic medium in the mouth. Octenidine dihydrochloride (OCT) is one such a product hypothesized to be capable of exerting beneficial in vitro and in vivo effects on cariogenic bacteria.[8,9]

Slee and O'Connor et al.[8] selected OCT (Sterling-Winthrop Research Institute, Rensselaer, NY, USA) from a series of bispyridine analogues that had been screened for antimicrobial activity against *S. mutans, Streptococcus sanguis, Actinomyces viscosus*, and *Actinomyces naeslundii* for their study. These investigators found octenidine to exhibit durability, and identified the minimum inhibitory concentration (MIC) as being 0.05 mg/ml in an aqueous solution. In rats, Shern et al.[9] showed that 0.5% OCT was almost as effective in decreasing plaque scores as 0.5% chlorhexidine gluconate (CHX), while Emilson et al.[10] found that 1% OCT reduced plaque scores in monkeys significantly better than 1% CHX.

Because both the absolute (compared to physiological saline (PS)) and relative (compared with CHX and polyvinylpyrrolidone—iodine (PVP—I)) antibacterial effects of OCT in saliva have already been established, the current study was conducted to assess the short-term (up to 120 min) effect of the newly developed Octenisept® (Schülke & Mayr, Norderstedt, Germany) containing 0.1% OCT, on total salivary bacterial count and cariogenic bacteria, mainly *S. mutans* and oral *Lactobacillus* species, in orthodontically treated patients. This is because salivary cariogenic bacteria are one of the initiators of enamel decalcification and smooth surface caries in orthodontic patients.[10] In addition, its effect was compared with the more commonly used CHX (0.2% chlorhexidine gluconate) and with PVP—I containing 7.5% poly(1-vinyl-2-pyrrolidone) iodine as its active ingredient.

Materials and methods

Experimental design

The absolute and relative antibacterial activities of OCT against total and cariogenic bacteria (*S. mutans* and *Lactobacillus* species) in saliva were studied both in vitro and in vivo.

In vitro tests

The in vitro effect of the mouth rinsing solutions was determined by the MIC of each rinsing solution with *S. mutans* (ATCC 27351/27951), *Streptococcus salivarius* (ATCC 25975), and *Lactococcus lactis* subspecies *lactis* strains, studied with a broth dilution method. Serial dilutions of 0.1% OCT, 0.2% CHX, and 7.5% PVP—I were prepared in trypticase soy broth. An overnight inoculum of 0.1 ml *S. mutans*, *S. salivarius*, and *Lactococcus lactis* subspecies *lactis* strains adjusted to 0.5 McFarland turbidity were added to each test tube. The test tubes were incubated at 37 °C overnight in a 5% CO_2 atmosphere for the first two strains and in anaerobic conditions for the latter. After the incubation period, a quantity of 0.001 ml was taken from each test tube and inoculated on a blood agar plate surface for the observation of any bacterial growth. In addition, the colony count was measured after an overnight incubation.

In vivo tests

The sample consisted of 18 patients (five male) aged 12 to 16 years selected from patients who were starting their orthodontic treatment at the Department of Orthodontics, Suleyman Demirel University, Isparta, Turkey. The research project was approved by the Ethics Committee of Suleyman Demirel University Medical Faculty. Written informed consent was obtained from each subject on a voluntary basis, with the option that the participants could withdraw from the study for any reason at any time, without affecting their orthodontic treatment. All patients were scheduled for full-bonded edgewise treatment with metal brackets on their anterior teeth and premolars and bands on their molars with 0.014 NiTi arc wire for leveling. None of the subjects had received antibiotics or topical antiseptics during the previous 30 days or had systemic diseases that could alter the amount or composition of their plaque or saliva. The study began the day after bonding the brackets and bands and lasted for 2 months with follow-up at 2-week intervals.

The volunteers were asked to collect 2—3 ml of unstimulated saliva in the morning immediately before the mouth rinse application as a baseline sample, and again at 15, 30, 60, and 120 min after one of the mouth rinse solutions had been used for 30 seconds. The volunteers were asked to brush their teeth the day before, after dinner, without toothpaste and to avoid eating breakfast and brushing in the morning. During the course of the experiment the volunteers were asked to refrain from drinking and eating between 9 and 11 a.m. One of the three mouth rinse solutions and a PS solution as control were applied for 30 seconds at each application (as per Lundstrom and Krasse[11]). The solutions used were: Octenisept (Schülke & Mayr, Norderstedt, Germany), Klorhex (Drogsan, Ankara, Turkey), and Batticon (Adeka, Samsun, Turkey), and a PS solution.

The experiment was performed on the same patient for each mouth rinse solution separately at 2-week intervals. At the beginning of each phase, the plaque scores of all subjects were brought to approximately identical levels with a thorough dental prophylaxis. A dental prophylaxis

was completed before the next mouth rinse solution was used. Thus, a week interval was applied between rinses.

All the samples were taken to and processed at the microbiology laboratory of the medical faculty in sterile conditions within 30 min, and stored at +4 °C until processing. After vortexing, a 10 μl aliquot of each saliva sample was diluted 1:10 000 with PS. The dilutions were established by our preliminary study in which suitable dilution ratios of the saliva samples for observing countable colony-forming units (cfu) on agar plates were determined. The original data, measured in cfu, were transformed to log(10) for statistical analysis and reported as log(cfu).

Mitis salivarius agar (Difco Laboratories, MD, USA) supplemented with 15% sucrose and bacitracin (0.2 U per ml of medium; MS-SB) and Rogosa agar (Oxoid, Basingstoke, Hampshire, UK), which are selective media for *S. mutans*[12] and for lactobacilli, respectively, were used.[13] Total viable counts of all cultivable facultative anaerobic and anaerobic bacteria were made on trypticase soy agar enriched with 5% (v/v) sheep blood.

Incubation of the samples was performed in an anaerobic atmosphere at 37 °C for 48 hours. After incubation, the total cfu on each plate were counted. Plates selectively inoculated for *S. mutans* and *Lactobacillus* species were incubated in the same gaseous mixture, then allowed to develop at room temperature for 24 hours to enhance colony development. Total viable counts for each bacterial species were morphotyped. Fine opaque, pale yellow colonies on Rogosa agar with Gram-positive, non-spore forming, and catalase-negative rods were further confirmed by positive indole and negative motility tests, and were considered to be *Lactobacillus* species.[14] Colonies growing on MS-SB agar with catalase-negative and Gram-positive cocci in chains on microscopy were considered to be *S. mutans*.

Statistical analysis

The statistical analyses were performed using SPSS (Windows version 12.0; Chicago, USA) software. The values of repeated bacterial count (cfu/ml) after PS, OCT, CHX, and PVP—I mouth rinsing were expressed as the minimum (min), maximum (max), and median. The bacterial count differences in median ranks for repeated measures among time intervals (before mouth rinsing (T0), 15-min effect (T1), 30-min effect (T2), 60-min effect (T3), 120-min effect (T4)) were compared by the non-parametric Friedman test, and significance levels were set at 0.05. The Wilcoxon signed ranks test was used to compare two dependent non-parametric values, and the significance level was chosen as 0.01, according to Bonferroni adjustment. The Friedman test was used to determine the bacterial count difference at each time interval for the different mouth rinsing solutions, and the Wilcoxon sign rank test was used for two dependent non-parametric values. Again, a significance level of 0.01 was chosen.

Results

In vitro results

S. mutans was completely inhibited with the 0.15 mg/ml PVP—I complex, 0.5 mg/ml CHX, and 0.1 mg/ml OCT concentrations, while *S. salivarius* was inhibited by the 0.15 mg/ml PVP—I, 2 mg/ml CHX, and 0.8 mg/ml OCT concentrations (Table 1). In addition, *Lactococcus lactis* subspecies *lactis* was completely inhibited at every tested concentration of OCT and CHX, but it grew at every concentration of the PVP—I complex tested. For this reason new concentrations were evaluated in a second stage for the antiseptic mouth rinses with *Lactococcus lactis* subspecies *lactis*. The results are shown in Table 2. Higher concentrations of PVP—I and lower concentrations of OCT and CHX were evaluated to determine the MIC of these solutions for *Lactococcus lactis* subspecies *lactis* (Table 2). MIC values of OCT, PVP—I, and CHX were determined as 0.003, 30, and 0.006 mg/ml, respectively, for *Lactococcus lactis* subspecies *lactis*.

The activities of CHX and OCT on *Lactococcus lactis* subspecies *lactis* were significantly higher when compared with their effects on *S. mutans* and *S. salivarius*. On the other

Table 1 In vitro antibacterial activities of octenidine dihydrochloride (OCT), polyvinylpyrrolidone—iodine (PVP—I), and chlorhexidine gluconate (CHX) for *Streptococcus mutans*, *Streptococcus salivarius*, and *Lactococcus lactis* subspecies *lactis*

	Concentration mg/ml	*Streptococcus mutans* cfu/ml	*Streptococcus salivarius* cfu/ml	*Lactococcus lactis* subspecies *lactis* cfu/ml
OCT	0.8	(−)	(−)	(−)
	0.4	(−)	1	(−)
	0.2	(−)	8	(−)
	0.1	(−)	20	(−)
	0.05	1	10^3	(−)
PVP—I	0.15	(−)	(−)	10^6
	0.075	2.10^2	2.10^2	4.10^6
	0.050	6.10^2	7.10^2	7.10^6
	0.025	10^3	10^3	10^7
	0.0125	6.10^4	7.10^3	2.10^7
CHX	2	(−)	(−)	(−)
	1	(−)	2.10^2	(−)
	0.5	(−)	4.10^2	(−)
	0.25	8.10^2	10^3	(−)
	0.125	1.10^3	6.10^3	(−)
Control		2.10^7	2.10^7	3.10^8

Table 2 In vitro antibacterial activities of octenidine dihydrochloride (OCT), polyvinylpyrrolidone—iodine (PVP—I), and chlorhexidine gluconate (CHX) for *Lactococcus lactis* subspecies *lactis*

	Concentration mg/ml	*Lactococcus lactis* subspecies *lactis* cfu/ml
OCT	0.01250	(−)
	0.00625	(−)
	0.00313	(−)
	0.00156	10^3
	0.00008	6.10^3
PVP—I	37.5	(−)
	30.0	(−)
	22.5	2.10^3
	15.0	10^4
	7.5	12.10^4
CHX	0.02500	(−)
	0.01250	(−)
	0.00625	(−)
	0.00313	8.10^3
	0.00156	2.10^4
Control		3.10^8

hand, very high concentrations of PVP—I were needed to inhibit the growth of *Lactococcus lactis* subspecies *lactis* when compared with other strains.

In vivo results

OCT had a significantly stronger impact on the microbial burden of the oral cavity than CHX beginning at 15 min post-application. PVP—I and CHX were significantly less effective compared with 0.1% OCT from the beginning to up to 120 min ($p < 0.01$). The antiseptic efficacy of 0.2% CHX on total bacteria and *Lactobacillus* species was very similar to the efficacy observed with 7.5% PVP—I mouth solution from the beginning of the antiseptic treatment to up to 120 min. In contrast, the antiseptic efficacy of 0.2% CHX on *S. mutans* was superior to the efficacy observed with the 7.5% PVP—I solution from the beginning of the antiseptic treatment to up to 120 min. The antimicrobial impact of CHX was very similar to PVP—I. Comparison of different mouth rinse solutions on antibacterial effects for each time interval and comparisons between the antiseptics are presented in Tables 3 and 4, respectively. Cfu—time graphs of total bacteria, *Lactobacillus* species, and *S. mutans* are presented in Figures 1, 2, and 3, respectively.

Discussion

The levels of cariogenic pathogens should be constantly reduced during orthodontic treatment. To achieve a fast reduction in cariogenic bacteria, primary dental care begins at home. Orthodontic treatment requires high levels of patient compliance; this is a problem especially in teenagers, an age group in which compliance is generally low. Practicing satisfactory oral hygiene, such as adequate tooth brushing, mouth rinsing, chewing gums, dental flossing, and using sustained release varnishes all play a vital role in maintaining healthy teeth throughout orthodontic treatment.[15—17]

However, only a few studies have been done with a novel oral antiseptic mouth rinse solution, OCT, to test its contribution to oral hygiene by determining its quantitative effects on *S. mutans*, *Lactobacillus* species, and total bacteria. For this reason, we aimed to determine the reduction or elimination of the cariogenic bacteria that cause smooth surface caries and white spots during orthodontic treatment, with this mouth rinse. Initially, we began detecting MIC activities of OCT, PVP—I, and CHX for *S. mutans* and *Lactobacillus* species, which are highly associated with caries.[18,19] Reduction of these bacteria from the oral cavity is a crucial

Table 3 Antibacterial effects of different mouth rinse solutions for each time interval

		Time[a]							
		T0—T1	T0—T2	T0—T3	T0—T4	T1—T2	T1—T4	T3—T4	T1—T3 T2—T3 T2—T4
Total bacteria	PS	NS	NS	NS	NS	NS	NS	NS	NS
	OCT	<0.001	<0.001	<0.001	<0.001	NS	0.007	0.002	NS
	CHX	<0.001	<0.001	<0.001	<0.001	<0.001	NS	NS	NS
	PVP—I	<0.001	NS	<0.001	<0.001	NS	NS	NS	NS
Lactobacillus species	PS	NS	NS	NS	NS	NS	NS	NS	NS
	OCT	<0.001	<0.001	<0.001	<0.001	NS	NS	NS	NS
	CHX	<0.001	<0.001	<0.001	<0.001	NS	NS	NS	NS
	PVP—I	NS	0.001	NS	NS	NS	NS	NS	NS
Streptococcus mutans	PS	NS	NS	NS	NS	NS	NS	NS	NS
	OCT	0.003	<0.001	<0.001	<0.001	NS	NS	NS	NS
	CHX	NS	<0.001	<0.001	0.001	NS	NS	NS	NS
	PVP—I	0.008	0.003	0.003	0.004	NS	NS	NS	NS

p-Values based on Wilcoxon test.
NS: not significant; PS: physiological saline; OCT: octenidine dihydrochloride; CHX: chlorhexidine gluconate; PVP—I: polyvinylpyrrolidone—iodine.
[a] T0: before mouth rinsing; T1: effect at 15 min; T2: effect at 30 min; T3: effect at 60 min; T4: effect at 120 min.

Table 4 Comparison of antibacterial effects of different mouth rinse solutions for each time

		Total bacteria	*Lactobacillus* species	*Streptococcus* mutans
Time 0	PS—OCT	NS	NS	NS
	PS—CHX	NS	NS	NS
	PS—PVP—I	NS	NS	NS
	OCT—CHX	NS	NS	NS
	OCT—PVP—I	NS	NS	NS
	CHX—PVP—I	NS	NS	NS
Time 1	PS—OCT	<0.001	<0.001	<0.001
	PS—CHX	NS	0.001	NS
	PS—PVPI	NS	0.007	NS
	OCT—CHX	<0.001	0.002	NS
	OCT—PVP—I	<0.001	0.006	NS
	CHX—PVP—I	NS	NS	NS
Time 2	PS—OCT	<0.001	<0.001	<0.001
	PS—CHX	<0.001	0.002	0.004
	PS—PVP—I	NS	0.002	0.004
	OCT—CHX	<0.001	<0.001	<0.001
	OCT—PVP—I	<0.001	<0.001	<0.001
	CHX—PVP—I	NS	NS	NS
Time 3	PS—OCT	<0.001	<0.001	<0.001
	PS—CHX	<0.001	<0.001	<0.001
	PS—PVP—I	0.007	0.005	0.006
	OCT—CHX	<0.001	0.006	<0.001
	OCT—PVP—I	<0.001	<0.001	<0.001
	CHX—PVP—I	NS	NS	NS
Time 4	PS—OCT	<0.001	<0.001	<0.001
	PS—CHX	0.001	<0.001	0.001
	PS—PVP—I	0.001	0.004	NS
	OCT—CHX	<0.001	<0.001	0.001
	OCT—PVP—I	<0.001	<0.001	0.001
	CHX—PVP—I	NS	0.009	NS

p-Values based on Wilcoxon tests.
NS: not significant; PS: physiological saline; OCT: octenidine dihydrochloride; CHX: chlorhexidine gluconate; PVP—I: polyvinylpyrrolidone—iodine.

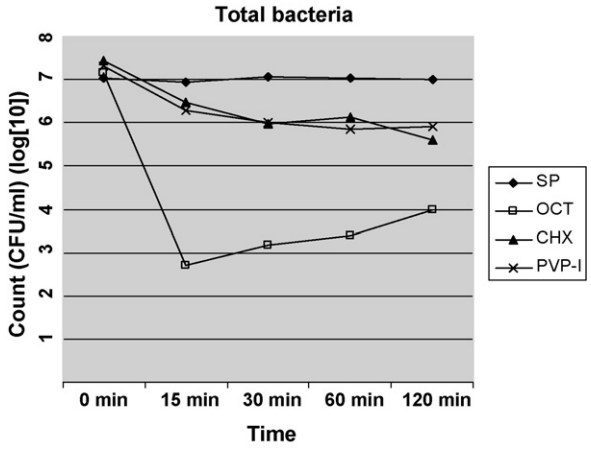


Figure 1 Cfu(log10)—time graph of total bacteria for 120 min after PS (physiological saline), OCT (octenidine dihydrochloride), CHX (chlorhexidine gluconate), and PVP—I (polyvinylpyrrolidone—iodine) treatment. The *p*-values for the mouth rinse solutions are as follows: PS: 0.914; OCT: <0.001; CHX: <0.001; PVP—I: <0.001.

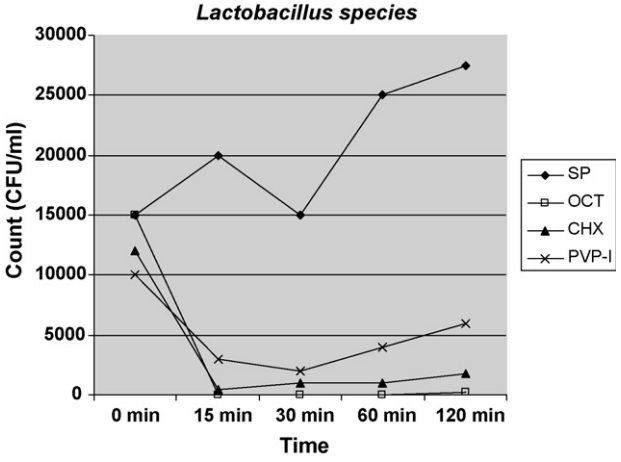


Figure 2 Cfu(log10)—time graph of *Lactobacillus* species for 120 min after PS (physiological saline), OCT (octenidine dihydrochloride), CHX (chlorhexidine gluconate), and PVP—I (polyvinylpyrrolidone—iodine) treatment. The *p*-values for the mouth rinse solutions are as follows: PS: 0.498; OCT: <0.001; CHX: <0.001; PVP—I: 0.003.

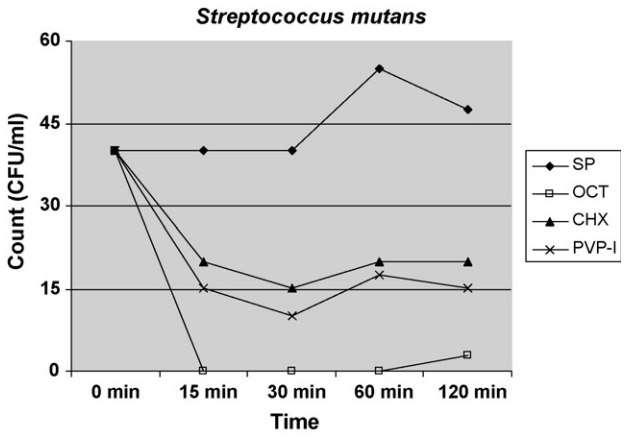


Figure 3 Cfu(log10)—time graph of *Streptococcus mutans* for 120 min after PS (physiological saline), OCT (octenidine dihydrochloride), CHX (chlorhexidine gluconate), and PVP—I (polyvinylpyrrolidone—iodine) treatment. The *p*-values for the mouth rinse solutions are as follows: SP: 0.018; OCT: <0.001; CHX: <0.001; PVP—I: <0.001.

step in the prevention and treatment of caries. It is not possible to predict the efficacy of a particular product if the antimicrobial activity is determined with in vitro methods exclusively.[20] Thus, the in vivo study model was designed to determine the absolute and relative short-term (two-hour) effect of OCT on *S. mutans*, *Lactobacillus* species, and total oral bacteria in orthodontically treated patients. The objective was to assess the relative antibacterial efficacy and short-term durability of each mouth rinse solution. We chose CHX as one of the rinses; CHX is considered to be the gold standard because of its antibacterial effect already determined in previous studies.[21—23]

Even though several other studies have tested the effects of CHX[24—26] and PVP—I,[27—29] the number of total and cariogenic bacteria in saliva after rinsing with OCT has not been tested before on orthodontically treated patients. OCT was found to be efficacious and compared favorably with CHX and PVP—I in its antibacterial effect in saliva. This effect continued for two hours. These results were consistent with those of Kramer et al.,[19] who reported that OCT and cetylpyridinium chloride were significantly more effective than other mouth rinsing solutions including Corsodyl® (which contains chlorhexidine gluconate) in their immediate value. These researchers reported that the only drawback of OCT use was its bitter taste.

The effect of mouth rinsing applications usually lasts 15—30 min. We detected the effect of antibacterial agents for up to 120 min, which Kramer et al. considered sufficient.[19] Both in vitro and in vivo results were consistent with these findings. A significant reduction in *S. mutans* and total bacterial counts occurred 15 min after rinsing with OCT when compared with PS ($p < 0.01$). However, the reduction was not significant when CHX and PVP—I mouth rinses were compared with PS after 15 min. Octenisept also showed the highest antibacterial effect after 120 min, while the antibacterial effect of CHX and PVP—I showed similarity at each time interval but they had lower benefits than OCT over the 120-min period. Both CHX and PVP—I were still effective after 120 min (Table 4).

Slee and O'Connor[8] found comparably favorable effects of OCT compared with CHX on *S. mutans*, *S. sanguis*, *A. viscosus*, and *A. naeslundii* with respect to overall antiplaque potency in vitro, whereas Samet et al.[30] found that the kinetics of OCT in killing *Staphylococcus aureus* depended on its concentration but was independent of bacterial genotype. Shern et al.[31] detected similar effects of these antibacterial agents in a rat and a monkey dental plaque model.[10] Our in vitro and in vivo study results support both of the above studies.

In the in vitro study of Pitten et al.,[32] PVP—I, OCT, and CHX caused a 10- to several-fold decrease in the number of total bacteria with different antiseptic concentrations and with different time intervals. *S. aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecium*, and *Candida albicans* were among the microorganisms examined. They found cetylpyridinium-based products to be less effective and used hydrogen peroxide as a control.

Beiswanger et al.[33] found that using 0.1% OCT yielded statistically significant lower levels of plaque, less gingivitis, and fewer bleeding sites when compared with the control product. The control product had a statistically significant higher stain formation and longer prophylaxis time to remove the stain. Thus, further studies must be performed for longer periods, and both the beneficial and adverse effects of OCT must be evaluated when comparing it with other antibacterial agents.

Conclusions

Three different mouth rinse solutions were compared with PS and with each other for their short-term antibacterial effects. From the tested rinsing solutions, OCT was found to be the most effective in substantially reducing total salivary and total cariogenic bacterial counts after 15, 30, 60, and 120 min ($p < 0.01$). This effect was found to be significantly different compared to PVP—I complex ($p < 0.01$), but not to CHX within these time intervals.

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Conflict of interest: No conflict of interest to declare.

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