Original Article

Microbiological Evaluation of Octenidine Dihydrochloride Mouth Rinse after 5 Days' Use in Orthodontic Patients

Alev Aksoy Dogan[a]; Emel Sesli Cetin[b]; Emad Hüssein[c]; Ali Kudret Adiloglu[d]

ABSTRACT

Objective: To determine the absolute and relative antibacterial activity of octenidine dihydrochloride (OCT) against total and cariogenic bacteria in saliva samples of patients with fixed orthodontic appliances during 5 days of usage.

Materials and Methods: The study group consisted of 5 male and 13 female subjects who were selected from patients in the Clinic of Orthodontics. Each patient was given physiologic saline (PS), chlorhexidine gluconate (CHX), polyvinylpyrrolidone-iodine complex (PVP-I), and OCT every morning for 5 days, each separated by a 2-week interval. Total and cariogenic bacteria in saliva samples of orthodontically treated patients with fixed appliances were collected during 5 days of usage. Unstimulated saliva was collected as a baseline sample. Saliva samples were collected at 15 minutes, and on the second, third, and fifth day after rinsing the mouth with any of the solutions for 30 seconds, and bacterial counts were detected.

Results: OCT showed an ultimate reduction of total viable oral bacteria, *Lactobacillus* species, and *Streptococcus mutans* in vivo. OCT also had a significantly greater inhibitory effect than 0.2% CHX and 7.5% PVP-I, from the beginning of the study until the fifth day after the orthodontic appliances were bonded ($P < .1$).

Conclusions: OCT compared favorably with respect to CHX and PVP-I complex in orthodontically treated patients with fixed appliances ($P \leq .1$). (*Angle Orthod.* 2009;79:766–772.)

KEY WORDS: Octenidine dihydrochloride; Antibacterial mouth rinse; *Streptococcus mutans*; *Lactobacillus*; Orthodontics

INTRODUCTION

The placement of fixed orthodontic appliances generally hinders good oral hygiene, and the appliance components can cause alterations in the oral microflora by reducing pH, increasing affinity of bacteria to the metallic surface because of electrostatic reactions, and causing retention areas for microorganisms. Thus, they lead to plaque accumulation around the bracket base.[1–3]

Increased levels of *Streptococcus mutans* and *Lactobacillus* species have been reported to be detected in the oral cavity after bonding orthodontic attachments, and several studies have reported that there is a positive correlation between dental caries and the degree of infection with *S mutans* and lactobacilli.[4–6] These microorganisms are known to cause alterations in its microenvironment that can be responsible for the enamel decalcification and smooth surface caries during orthodontic treatment.[7] An increase in the *S mutans* level will also result in an imbalance between demineralization and remineralization of dental hard tissue, which leads to the acute inflammation of gingiva,[8] so the reduction of cariogenic bacteria from the oral cavity is a crucial step for the prevention and treatment of caries during orthodontic treatment.

Practicing satisfactory oral hygiene, such as adequate tooth brushing, mouth rinsing, gum chewing,

[a] Assistant Professor, Department of Orthodontics, Faculty of Dentistry, Süleyman Demirel University, Isparta, Turkey.

[b] Assistant Professor, Department of Clinical Microbiology, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey.

[c] Assistant Professor, Chairman of the Department of Orthodontics, Faculty of Dentistry, Arab American University, Jenin, Palestine.

[d] Associate Professor, Department of Clinical Microbiology, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey.

Corresponding author: Dr Alev Aksoy Dogan, Department of Orthodontics, Süleyman Demirel University, Isparta, 32100 Turkey
(e-mail: alevak2000@yahoo.com)

Accepted: September 2008. Submitted: June 2008.

 DOI: 10.2319/062008-322.1

dental flossing, and using sustained-release varnishes, plays a vital role in maintaining healthy teeth throughout orthodontic treatment.[9–11] The effective control of dental plaque and thereby of dental diseases of microbial etiology by mechanical means is dependent on patient compliance and appears to be limited to the failure of the patient to reach certain plaque-infected sites that are hindered by the brackets and wires. Hence, a great need arises for the development of antibacterial and antiadhesive chemotherapeutic agents to assist in the control of infections associated with dental plaque.

Octenidine dihydrochloride (OCT) has been shown to be effective in controlling bacterial plaque formation.[12,13] In the study of Rosin et al,[14] the plaque regrowth and bacterial counts were evaluated at the end of the fifth day. The antibacterial and antiplaque effect of polyhexamethylene biguanide hydrochloride, chlorhexidine gluconate (CHX), and Listerine mouth rinses were evaluated after the tooth cleaning was ceased.[14] They reported that while the reduction of bacterial growth on teeth surfaces with 0.12% biguanide hydrochloride was significantly greater compared with the placebo or Listerine, chlorhexidine was more effective than biguanide hydrochloride after 5 days. In addition, chlorhexidine was significantly more effective in reducing bacterial counts on mucosa than biguanidine and Listerine after 5 days. Arweiler et al[15] assessed the bacterial vitality between 24 and 96 hours for two commercial 0.2% chlorhexidine solutions and compared them with a negative control. The results after 96 hours suggested that 0.2% alcohol-containing solution showed superiority in inhibiting plaque regrowth and reducing bacterial vitality compared with the solution with antidiscoloration system.

In our previously published study, we detected the MIC activities of OCT, polyvinylpyrrolidone-iodine complex (PVP-I), and CHX for *S mutans* and *Lactobacillus* species. Our results yielded that OCT had a significantly greater inhibitory effect on the studied bacteria than 0.2% CHX and 7.5% PVP-I from 15 minutes to 120 minutes following the application ($P < .01$).[16] In the present study, we aimed to determine the reduction effects of these mouth rinses, during their 5 days of usage time, on the cariogenic bacteria that cause acute gingivitis, smooth surface caries, and white spot lesions during orthodontic treatment, which leads to tooth and periodontal damage at the end of the treatment.

MATERIALS AND METHODS

The study group consisted of 18 patients (5 male, 13 female; 12 to 16 years old) selected from patients assigned for orthodontic treatment at the Department of Orthodontics, Süleyman Demirel University, Isparta, Turkey. The research project was approved by the Local Ethics Committee of Medical Faculty of Süleyman Demirel University. Written informed consent was obtained from each subject on a voluntary basis, with the option that the participants could withdraw for any reason at any time. All subjects were selected from the individuals who had good oral hygiene and whose plaque and bleeding scores were not different in the beginning (Table 1). All patients underwent full-bonded edgewise treatment with metal brackets on their anterior teeth and premolars and bands on their molars. None of the subjects received antibiotics or topical antiseptics during the previous 30 days or had systemic disease which would have altered the amount or composition of the plaque or saliva. There was no known hypersensitivity to any of the used mouth-rinsing antibacterial solutions.

Table 1. Plaque and Bleeding İndex Results Before Saliva Collection

	Index (Median)[a]	
	Plaque Index	Bleeding Index
Previous PS	0.10 (0–1.95)	0.15 (0–0.40)
Previous OCT	0.07 (0–1.05)	0.18 (0.04–0.53)
Previous CHX	0.02 (0–0.75)	0.12 (0–0.38)
Previous PVP-I	0.02 (0–0.85)	0.20 (0–0.45)
P value*	.210	.348

[a] PS indicates physiologic saline; OCT, octenidine dihydrochloride; CHX, chlorhexidine gluconate; and PVP-I, polyvinylpyrrolidone-iodine complex.

* Friedman test; test for several related samples.

The study began the day after bonding the brackets and bands and lasted 2 months by 2-week intervals. The experiment was done on the same patient for each mouth rinse solution separated by 2 consecutive two week intervals. Measures of plaque and papilla bleeding indexes were determined according to Sillness and Löe[17] at the beginning of each phase. The plaque and bleeding scores of all subjects were brought to zero with a thorough dental prophylaxis at baseline for each mouth-rinsing solution. Thus, a week interval was given to each patient for dental prophylaxis until the trial of the next mouth-rinsing solution. The solutions used for the experiments were OCT (Octenisept, Schülke & Mayr GmbH, Norderstedt, Germany), 0.2% CHX (Klorhex, Drogsan, Ankara, Turkey), 7.5% PVP-I (Batticon, Adeka, Samsun, Turkey), and physiologic saline (PS).

The volunteers were asked to collect 2–3 mL of unstimulated saliva in the morning as a baseline sample and at 15 minutes after swishing the mouth. They were also instructed to use 15 mL of the mouth rinse for 30 seconds once a day each morning for 5 days. The saliva collection was repeated on the second, third,

and fifth day. The volunteers were asked to brush their teeth after dinner without toothpaste the day before sample collection and refrain from eating in the morning and brushing during the course of the experiment until the end of the fifth day. They were asked not to have breakfast before giving saliva each morning. All samples were carried to the Microbiology Laboratory of the Medical Faculty under sterile conditions in half an hour's time and stored at +4°C until they were processed on the same day. Mitis Salivarius agar (Difco Laboratories, Sparks, Md) supplemented with 15% sucrose and bacitracin (0.2 U per mL of medium) for *S mutans*, and Rogosa agar (Oxoid, Basingstoke, Hampshire, England) for *Lactobacillus* species were used as selective mediums. Total viable counts of all cultivable facultative anaerobic bacteria were incubated onto Trypticase soy agar enriched with 5% (v/v) sheep blood. Microbiologic procedures were established according to Dogan et al.[16] Results were presented as colony-forming units (CFU)/mL.

Statistical Analysis

The statistical analyses were performed by using Statistical Package for Social Sciences (SPSS for Windows, Version 11.5, Chicago, Ill) 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 recorded values were transferred to log values before statistical analyses. The bacterial count differences in median ranks for repeated measures among time intervals (before mouth rinsing [T0], 15 minute effect [T1], second day effect [T2], third day effect [T3], and fifth day effect [T4]) were compared by the nonparametric Friedman test, and significance levels were set at .05. Wilcoxon signed rank test was used to compare two dependent nonparametric values, and significance level was chosen as .01 according to Bonferroni adjustment. The Friedman test was used to determine the bacterial count difference in each time interval for different mouth-rinsing solutions, and the Wilcoxon signed rank test was used for two dependent nonparametric values. Significance level was chosen as .01 according to Bonferroni adjustment. Briefly, the general level of significance was .05, and the local *P* values were adjusted according to Bonferroni due to multiple testing.

RESULTS

The minimum, maximum, and median CFU of total bacteria, *Lactobacillus* species and *S mutans* in the saliva of subjects before mouth rinsing (T0), on 15 minutes after swishing the mouth, and on the second, third, and fifth days during treatment with antiseptics are presented in Table 2. OCT had a significantly stronger impact on the microbial burden of the oral cavity than the other mouth-rinsing solutions from the beginning of the study until the fifth day ($P < .01$). The antiseptic efficacy of CHX 0.2% on *S mutans*, *Lactobacillus* species, and total flora was very similar to the efficacy observed with 7.5% PVP-I mouth solution from the beginning of the antiseptic treatment until the fifth day (Table 2).

CFU-time graphics of total bacteria, *Lactobacillus* species, and *S mutans* are presented in Figures 1, 2, and 3. Bacterial count changes detected with different mouth rinses for each time period are presented in Table 3. In addition, a comparison of the antibacterial effect of different mouth rinse solutions for each time is given in Table 4. There was no significant difference between bacterial counts detected in the saliva of subjects before mouth rinsing. We have not detected significant difference between antibacterial effects of CHX and PVP-I for any time period. On the other hand, OCT displayed significantly more antibacterial effect on all time periods except for time period T1 for *S mutans*.

DISCUSSION

The accumulation of supragingival dental plaque on tooth surfaces is the major etiological component in the development of caries and periodontal disease. Numerous reports have documented that the flora change from primarily Gram-positive to Gram-negative bacteria in conditions leading to gingivitis.[18,19] When mechanical plaque control is hampered, the chemical procedure remains the next best choice. Even though several other studies have tested the effects of CHX[20–22] and PVP-I,[23] there are only a few studies that tested OCT[24–28] to evaluate its contribution to oral hygiene by determining its effects on the number of total and cariogenic bacteria during its usage time.

In this study, *S mutans* was reduced significantly with CHX ($P < .05$) on T1 and T2 time periods and began to increase on time period T3. Significant reduction in *S mutans* levels was similar to the results of studies in which CHX varnish[10,29–32] and mouth rinse[20–23] were used, but the increase in *S mutans* count on the third day opens the lasting antibacterial effect of CHX to debate.

Decker et al[33] reported that among all antiseptic formulations (Olaflur, CHX) the application of OCT had the strongest impact in terms of lethal effect on the attached bacteria. In particular, the drastic $\log_{10}$ reduction of CFU values in the range of 8 belonging to the antibacterial effect of OCT resembles the findings of our previous study.[16] In our study, OCT was found to be efficacious and compared favorably with CHX and

Table 2. The Minimum, Maximum, and Median CFU (log10) of Total Bacteria, *Lactobacillus* Species and *Streptoccocus mutans* in the Saliva of Subjects During Treatment With Different Antiseptics

Solution[a]-Time[b]	Total[c]			*Lactobacillus* species[c]			*Streptococcus mutans*[c]		
	Min	Max	Median	Min	Max	Median	Min	Max	Median
PS-T0	5.30	6.60	7.04	2.85	4.78	4.18	1.00	2.30	1.60
PS-T1	5.00	7.60	6.95	2.78	4.60	4.30	1.00	3.15	1.60
PS-T2	6.00	7.78	7.00	3.00	4.78	4.09	1.00	2.60	1.78
PS-T3	6.00	7.78	7.08	3.00	4.85	4.35	1.00	3.60	1.81
PS-T4	5.30	7.85	7.09	3.00	4.85	4.44	1.00	2.65	1.88
* *P* value		.041			.042			<.001	
OCT-T0	5.78	7.60	7.13	2.78	4.78	4.18	1.00	2.30	1.60
OCT-T1	NG	6.30	2.70	NG	3.70	NG	NG	2.60	NG
OCT-T2	NG	5.00	3.90	NG	3.00	NG	NG	1.18	0.40
OCT-T3	NG	5.30	3.00	NG	3.30	NG	NG	1.18	0.54
OCT-T4	NG	5.48	3.40	NG	3.00	0	NG	1.30	0.60
* *P* value		<.001			<.001			<.001	
CHX-T0	5.70	7.60	7.44	2.78	4.60	4.08	1.30	2.00	1.60
CHX-T1	5.30	7.48	6.48	1.00	4.48	2.60	NG	1.90	1.30
CHX-T2	4.00	6.30	5.40	2.00	4.00	3.00	0.30	1.78	1.35
CHX-T3	4.00	6.48	5.30	2.00	4.00	2.78	0.60	1.90	1.60
CHX-T4	4.30	6.70	5.54	2.00	4.00	3.00	0.48	2.00	1.54
* *P* value		<.001			<.001			.025	
PVP-I-T0	5.70	7.65	7.30	3.30	4.60	4.00	1.00	2.30	1.60
PVP-I-T1	4.70	7.18	6.30	2.00	4.48	3.48	0	2.00	1.18
PVP-I-T2	5.00	7.00	5.60	2.00	4.00	3.08	0.30	1.78	1.04
PVP-I-T3	5.30	7.18	5.70	2.00	4.00	3.30	0	1.70	1.00
PVP-I-T4	5.00	7.30	5.48	2.00	4.30	3.18	0	1.70	1.18
* *P* value		<.001			<.001			.004	

[a] PS indicates physiologic saline; OCT, octenidine dihydrochloride; CHX, chlorhexidine gluconate; and PVP-I, polyvinylpyrrolidone-iodine complex.

[b] T0: before mouth rinsing; T1: 15 minutes after swishing the mouth; T2: second day effect; T3: third day effect; and T4: fifth day effect.

[c] NG indicates no growth.

* Friedman test.

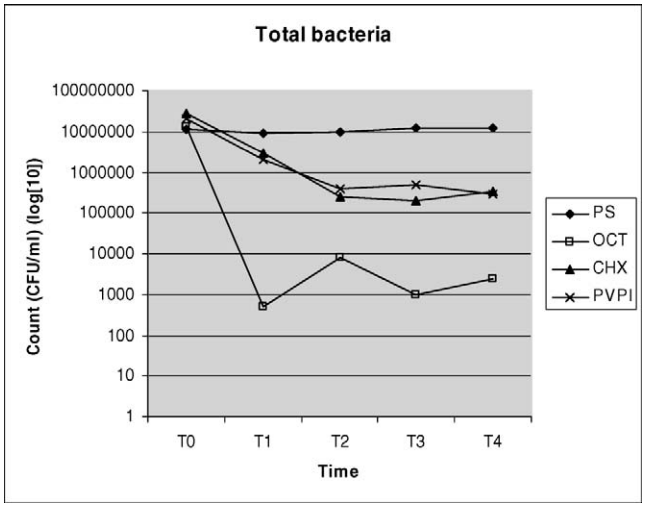

Figure 1. CFU (log10)–time graphic of total bacteria for 5 days after PS (physiologic saline), OCT (octenidine dihydrochloride), CHX (chlorhexidine gluconate), and PVP-I (polyvinylpyrrolidone–iodine) treatment. The *P* values for the mouth rinse solutions are: PS: not significant; OCT: <.001; CHX: <.001; PVP-I: <.001.

PVP-I in its antibacterial effect in saliva. This effect was seen on the third and fifth days as well. These results were also consistent with those of Kramer et al[28] 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. Beiswanger et al[13] also have reported that the group rinsing with 0.1% OCT, had significantly lower levels of plaque, less gingivitis, and fewer bleeding sites when compared with the control group.

Slee and O'Connor[34] found comparably favorable effects of OCT compared with CHX on *S mutans*, *Streptococcus sanguis*, *Actinomyces viscosus*, and *Actinomyces naeslundii* with respect to overall antiplaque potency in vitro, whereas Samet et al[35] found that the kinetics of OCT in killing *Staphylococcus aureus* depended on its concentration but was independent of bacterial genotype. Sedlock and Bailey[36] also established the therapeutic superiority of this antiseptic agent to CHX. In the 4-week follow-up study of Sari and Birinci,[22] it was reported that *S mutans* and lac-

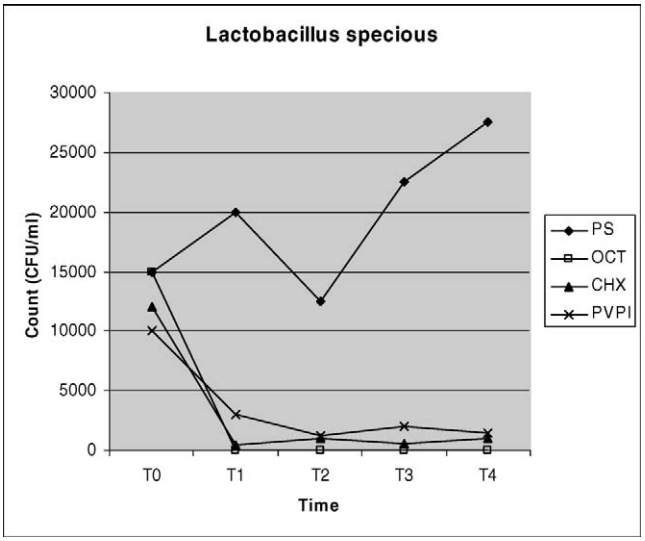

Figure 2. CFU (log10)–time graph of *Lactobacillus* species for 5 days after PS (physiologic saline), OCT (octenidine dihydrochloride), CHX (chlorhexidine gluconate), and PVP-I (polyvinylpyrrolidone-iodine) treatment. The *P* values for the mouth rinse solutions are: PS: not significant; OCT: <.001; CHX: <.001; PVP-I: .003.

tobacilli levels increased significantly after bonding the fixed appliances; however, even a low concentration of 0.2% CHX mouth rinse significantly reduced *S mutans* levels. They suggested that 0.2% CHX mouth rinse could successfully be used to decrease *S mutans* levels in combination with tooth brushing and flossing. In our study, we evaluated the antibacterial effect of CHX according to Rosin et al[14] and Arweiler et al[15] without tooth brushing and determined that OCT was found to be efficacious and compared favorably with CHX and PVP-I in its antibacterial effect in saliva during the 5 days of usage. *Lactobacillus* species decreased significantly by the usage of the three different

Table 4. Comparison of the Antibacterial Effect of Different Mouth Rinse Solutions for Each Time Period[a]

		Total Bacteria	*Lactobacillus* Species	*Streptococcus mutans*
T0	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
T1	PS-OCT	<.001	<.001	<.001
	PS-CHX	NS	.001	NS
	PS-PVP-I	NS	.007	NS
	OCT-CHX	<.001	.002	NS
	OCT-PVP-I	<.001	.006	NS
	CHX-PVP-I	NS	NS	NS
T2	PS-OCT	<.001	<.001	<.001
	PS-CHX	<.001	<.001	.001
	PS-PVP-I	<.001	.001	.003
	OCT-CHX	<.001	<.001	<.001
	OCT-PVP-I	<.001	<.001	.002
	CHX-PVP-I	NS	NS	NS
T3	PS-OCT	<.001	<.001	<.001
	PS-CHX	<.001	<.001	.001
	PS-PVP-I	<.001	.001	<.001
	OCT-CHX	.001	.003	.001
	OCT-PVP-I	<.001	<.001	NS
	CHX-PVP-I	NS	NS	NS
T4	PS-OCT	<.001	<.001	<.001
	PS-CHX	<.001	<.001	<.001
	PS-PVP-I	<.001	.001	<.001
	OCT-CHX	.003	<.001	<.001
	OCT-PVP-I	.002	<.001	.003
	CHX-PVP-I	NS	NS	NS

[a] T0: before mouth rinsing; T1: 15 minutes after swishing the mouth; T2: second day effect; T3: third day effect; T4: fifth day effect.
P values based on Wilcoxon test. NS indicates not significant according to $P < .01$***.

Table 3. Bacterial Count Changes Detected With Different Mouth Rinses[a] for Each Time Period*

		Time[b]									
		T0–T1	T0–T2	T0–T3	T0–T4	T1–T2	T1–T3	T1–T4	T2–T3	T2–T4	T3–T4
Total bacteria	PS	NS	NS	NS	NS	NS	NS	NS	NS	.007	NS
	OCT	<.001	<.001	<.001	<.001	NS	NS	NS	NS	NS	NS
	CHX	<.001	<.001	<.001	<.001	.001	.002	.006	NS	NS	NS
	PVP-I	<.001	<.001	<.001	<.001	NS	NS	NS	NS	NS	NS
Lactobacillus species	PS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	OCT	<.001	<.001	<.001	<.001	NS	NS	NS	NS	NS	NS
	CHX	<.001	<.001	<.001	<.001	NS	NS	NS	NS	NS	NS
	PVP-I	.001	.001	.001	.003	NS	NS	NS	NS	NS	NS
Streptococcus mutans	PS	NS	.001	<.001	<.001	NS	NS	NS	NS	.009	NS
	OCT	.003	<.001	<.001	<.001	NS	NS	NS	NS	NS	NS
	CHX	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	PVP-I	.008	.003	.001	.001	NS	NS	NS	NS	NS	NS

[a] PS indicates physiologic saline; OCT, octenidine dihydrochloride; CHX, chlorhexidine gluconate; and PVP-I, polyvinylpyrrolidone-iodine complex.
[b] T0: before mouth rinsing, T1: 15 minutes after swishing the mouth; T2: second day effect; T3: third day effect; T4: fifth day effect.
* *P* values based on Wilcoxon test. NS indicates not significant according to $P < .01$.

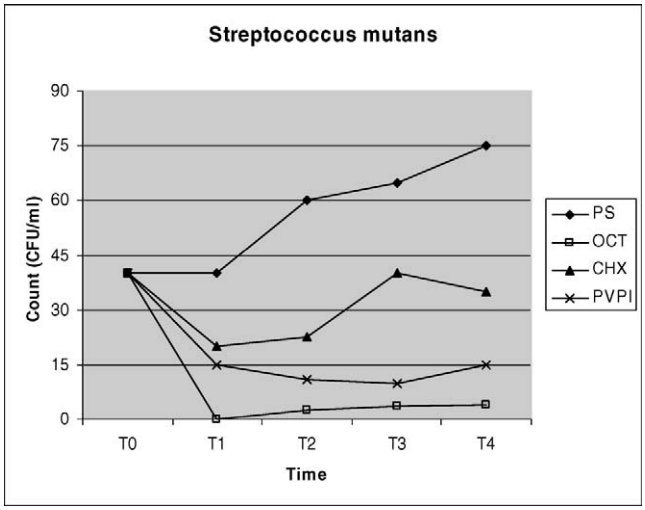

Figure 3. CFU (log10)–time graph of *Streptococcus mutans* for 5 days after PS (physiologic saline), OCT (octenidine dihydrochloride), CHX (chlorhexidine gluconate), and PVP-I (polyvinylpyrrolidone-iodine) treatment. The *P* values for the mouth rinse solutions are: PS: $<.001$; OCT: $<.001$; CHX: not significant; PVP-I: .001.

antibacterial mouth-rinsing solutions. *S mutans* levels were significantly decreased by OCT and PVP-I until the fifth day but began to increase during CHX usage in the third day. Thus, we can suggest that while OCT can be selected alone for the immediate eradication of acute gingival inflammation, CHX might be more efficient in combination with mechanical plaque removal, such as tooth brushing, in controlling chronic inflammation related to the increment of *S mutans* and lactobacilli levels in orthodontically treated patients with fixed appliances, and for the prevention of enamel decalcification and white spot lesions.

CONCLUSIONS

- During 5 days of usage, OCT was the most effective mouth rinse among the tested rinses as evidenced by a substantial reduction of total salivary and cariogenic bacterial counts ($P < .01$).
- Although we can suggest that patients can use OCT mouth rinse once a day during orthodontic treatment, recommendation of OCT for long-term use in orthodontic patients is not justified because some unanswered questions still remain.

ACKNOWLEDGMENT

We especially thank Associate Professor Ersin Uskun for her professional assistance with statistical evaluations.

REFERENCES

1. Türkkahraman H, Sayin Ö, Bozkurt FY, Yetkin Z, Kaya S, Önal S. Archwire ligation techniques, microbial colonization, and periodontal status in orthodontically treated patients. *Angle Orthod.* 2005;75:227–232.
2. Brusca MI, Chara O, Sterin-Borda L, Rosa AC. Influence of different orthodontic brackets on adherence of microorganisms *in vitro*. *Angle Orthod.* 2007;77:331–336.
3. Ahn SJ, Lee SJ, Lim BS, Nahm DS. Quantitative determination of adhesion patterns of cariogenic streptococci to various orthodontic brackets. *Am J Orthod Dentofacial Orthop.* 2007;132:815–821.
4. Lundstrom F, Krasse B. *Streptococcus mutans* and lactobacilli frequency in orthodontic patients; the effect of chlorhexidine treatments. *Eur J Orthod.* 1987;9:109–116.
5. Lundstrom F, Krasse B. Caries incidence in orthodontic patients with high levels of *Streptococcus mutans*. *Eur J Orthod.* 1987;9:117–121.
6. Hallgren A, Oliveby A, Twetman S. Caries associated microflora in plaque. *Scand J Dent Res.* 1992;100:140–143.
7. Mitchell L. Decalcification during orthodontic treatment with fixed appliances—an overview. *Br J Orthod.* 1992;19:199–205.
8. Balenseifen JW, Madonia JV. Study of dental plaque in orthodontic patients. *J Dent Res.* 1970;49:320–324.
9. Aksoy A, Duran N, Toroglu S, Koksal F. Short-term effect of mastic gum on salivary concentration of cariogenic bacteria in orthodontic patients. *Angle Orthod.* 2007;77:124–128.
10. Beyth N, Redlich M, Harari D, Friedman M, Steinberg D. Effect of sustained-release chlorhexidine varnish on *Streptococcus mutans* and *Actinomyces viscosus* in orthodontic patients. *Am J Orthod Dentofacial Orthop.* 2003;123:345–348.
11. Isotupa KP, Gunn S, Chen CY, Lopatin D, Mäkinen KK. Effect of polyol gums on dental plaque in orthodontic patients. *Am J Orthod Dentofacial Orthop.* 1995;107:497–504.
12. Emilson CG, Bowen WH, Robrish SA, Kemp CW. Effect of the antibacterial agents octenidine and chlorhexidine on the plaque flora in primates. *Scand J Dent Res.* 1981;89:384–392.
13. Beiswanger BB, Mallatt ME, Mau MS, Jackson RD, Hennon DK. The clinical effects of a mouth rinse containing 0.1% octenidine. *J Dent Res.* 1990;69:454–457.
14. Rosin M, Welk A, Kocher T, Majic-Todt A, Kramer A, Pitten FA. The effect of a polyhexamethylene biguanide mouthrinse compared to an essential oil rinse and a chlorhexidine rinse on bacterial counts and 4-day plaque regrowth. *J Clin Periodontol.* 2002;29:392–399.
15. Arweiler NB, Boehnke N, Sculean A, Hellwig E, Auschill TM. Differences in efficacy of two commercial 0.2% chlorhexidine mouthrinse solutions: a 4-day plaque re-growth study. *J Clin Periodontol.* 2006;33:334–339.
16. Dogan AA, Adiloglu AK, Önal S, Çetin ES, Polat E, Uskun E, Köksal F. Short-term relative antibacterial effect of octenidine dihydrochloride on the oral microflora in orthodontically treated patients. *Int J Infect Dis.* 2008;12:19–25.
17. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand.* 1964;22:121–135.
18. Moore WE, Holdeman LV, Smibert RM, Cato EP, Burmeister JA, Palcanis KG, Ranney RR. Bacteriology of experimental gingivitis in children. *Infect Immun.* 1984;46:1–6.
19. Kara C, Demir T, Tezel A, Zihni M. Aggressive periodontitis with streptococcal gingivitis: a case report. *Eur J Dent.* 2007;1:251–255.
20. Anderson GB, Bowden J, Morrison EC, Caffesse RG. Clinical effect of chlorhexidine mouthwashes on patients undergoing orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 1997;111:606–612.
21. Brightman LJ, Terezhalmy GT, Greenwell H, Jacobs M, En-

low DH. The effects of a 0.12% chlorhexidine gluconate mouth rinse on orthodontic patients aged 11 through 17 with established gingivitis. *Am J Orthod Dentofacial Orthop.* 1991;100:6324–6329.
22. Sari E, Birinci I. Microbiological evaluation of 0.2% chlorhexidine gluconate mouth rinse in orthodontic patients. *Angle Orthod.* 2007;77:881–884.
23. Demir A, Malkoç S, Şengün A, Koyutürk AE, Şener Y. Effects of chlorhexidine and povidone-iodine mouth rinses on the bond strength of an orthodontic composite. *Angle Orthod.* 2004;75:392–396.
24. Ghannoum MA, Elteen AK, Ellabib M, Whittaker PA. Antimycotic effects of octenidine and pirtenidine. *J Antimicrob Chemother.* 1990;25:237–245.
25. Pitten FA, Werner HP, Kramer A. A standardized test to assess the impact of different organic challenges on the antimicrobial activity of antiseptics. *J Hosp Infect.* 2003;55: 108–115.
26. Pitten FA, Kramer A. Antimicrobial efficacy of antiseptic mouthrinse solutions. *Eur J Clin Pharmacol.* 1999;55:95–100.
27. Smith RN, Andersen RN, Kolenbrander PE. Inhibition of intergeneric coaggregation among oral bacteria by cetylpyridinium chloride, chlorhexidine digluconate and octenidine dihydrochloride. *J Periodontal Res.* 1991;26:422–428.
28. Kramer A, Hoppe H, Krull B, Pitten FA, Rosenau S. Antiseptic efficacy and acceptance of Octenisept computed with common antiseptic mouthwashes [in German]. *Zentralbl Hyg Umweltmed.* 1998;200:443–456.
29. Derks A, Frencken J, Bronkhorst E, Kuijpers-Jagtman AM, Katsaros C. Effect of chlorhexidine varnish application on mutans streptococci counts in orthodontic patients. *Am J Orthod Dentofacial Orthop.* 2008;133:435–439.
30. Jenatschke F, Elsenberger E, Welte HD, Schlagenhauf U. Influence of repeated chlorhexidine varnish applications on mutans streptococci counts and caries increment in patients treated with fixed orthodontic appliances. *J Orofac Orthop.* 2001;62:36–45.
31. Madléna M, Vitalyos G, Márton S, Nagy G. Effect of chlorhexidine varnish on bacterial levels in plaque and saliva during orthodontic treatment. *J Clin Dent.* 2000;11:42–46.
32. Attin R, Tuna A, Attin T, Brunner E, Noack MJ. Efficacy of differently concentrated chlorhexidine varnishes in decreasing Mutans streptococci and lactobacilli counts. *Arch Oral Biol.* 2003;48:503–509.
33. Decker EM, Weiger R, Wiech I, Heide PE, Brecx M. Comparison of antiadhesive and antibacterial effects of antiseptics on *Streptococcus sanguinis*. *Eur J Oral Sci.* 2003;111: 144–148.
34. Slee AM, O'Connor JR. In vitro antiplaque activity of octenidine dihydrochloride (WIN 41464-2) against preformed plaques of selected oral plaque-forming microorganisms. *Antimicrob Agents Chemother.* 1983;23:3:379–384.
35. Samet A, Bronk M, Kur J, Juszczyk J. Antimicrobial effectiveness of the Octanisept disinfectant against methicillin-resistant *Staphylococcus aureus* strains. *Clin Microbiol Infect.* 2000;6(suppl 1):111.
36. Sedlock DM, Bailey DM. Microbicidal activity of octenidine hydrochloride, a new alkanediylbis (pyridine) germicidal agent. *Antimicrob Agents Chermother.* 1985;28:786–790.