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MPC Van Leeuwen
NAM Rosema
PA Versteeg
DE Slot
AJ Van Winkelhoff
GA Van der Weijden

Authors' affiliations:
MPC Van Leeuwen, *NAM Rosema*, *PA Versteeg*, *DE Slot* *GA Van der Weijden*, Department of Periodontology, Academic Center for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, Amsterdam, The Netherlands
MPC Van Leeuwen, Clinic for Dentistry, Bussum, The Netherlands
AJ Van Winkelhoff, Department of Dentistry and Oral Hygiene, University of Groningen, Groningen, The Netherlands
AJ Van Winkelhoff, Department of Medical Microbiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
GA Van der Weijden, Clinic for Periodontology, Utrecht, The Netherlands

Correspondence to:
D. E. Slot
Department of Periodontology
Academic Centre for Dentistry Amsterdam (ACTA)
University of Amsterdam and VU University Amsterdam
Gustav Mahlerlaan 3004
1081 LA Amsterdam
The Netherlands
Tel.: +31 20 5980 307
E-mail: d.slot@acta.nl

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Van Leeuwen MPC, Rosema NAM, Versteeg PA, Slot DE, Van Winkelhoff AJ, Van der Weijden GA. Long-term efficacy of a 0.07% cetylpyridinium chloride mouth rinse in relation to plaque and gingivitis: a 6-month randomized, vehicle-controlled clinical trial.

Long-term efficacy of a 0.07% cetylpyridinium chloride mouth rinse in relation to plaque and gingivitis: a 6-month randomized, vehicle-controlled clinical trial

Abstract: *Objective:* To evaluate the effectiveness of 0.07% cetylpyridinium chloride (CPC) mouth rinse for reduction of gingival inflammation and inhibition of plaque compared to a vehicle control (VC) mouth rinse over a 6-month period. *Materials & Methods:* Participants (n = 62) used their randomly assigned product as adjunct to toothbrushing. Bleeding, plaque and staining scores were assessed at baseline, 3 and 6 months. Plaque and saliva samples were taken at each assessment monitoring possible shifts in the composition of the microbiota. *Results:* A significant difference (P = 0.002) in favour of the CPC mouth rinse, with respect to plaque scores, was found. Bleeding scores at 6 months were not significantly different (P = 0.089). However, when correcting for baseline values, a tendency towards a significant difference in bleeding scores at end trail was observed in favour of the CPC mouth rinse (P = 0.061). Regarding staining at 3 and 6 months, a small but significant difference (8.6% and 10.4%, respectively) (P < 0.0001) was observed with lower scores for the VC group. There was a significant reduction in total anaerobic count in the CPC group at 6 months (P < 0.05). The ratio of aerobes/anaerobes was markedly increased at 3 months, especially in the CPC group. No further differences were observed between groups at 6 months. *Conclusions:* The use of 0.07% CPC mouth rinse was significantly more effective in reducing plaque scores than the vehicle control. Bleeding scores were not different at 6 months. The test product was well accepted and did not cause any serious clinical side effects or negatively affected the microbiota.

Key words: cetylpyridinium chloride; clinical trial; dental plaque; gingivitis; mouth rinse

Introduction

Micro-organisms in the oral cavity grow in complex biofilms on hard and soft tissues. Dental plaque, however, is a multispecies biofilm of micro-organisms that grows on hard tissues only. The efficient removal of dental plaque is essential for maintaining oral health, as plaque has long been identified as a critical factor in the aetiology of caries, gingival inflammation and chronic periodontitis (1–3). Toothbrushing is generally accepted as the most efficient oral hygiene method of cleaning one's teeth. However, a recent systematic review assessing the efficacy in dental plaque removal showed that following a single brushing exercise, the

plaque reduction is 42% on average (4). Patients' efforts, however, are often compromised by the presence of hard-to-reach areas as well as inadequate skill, poor motivation and lack of compliance. Consequently, the use of antimicrobial mouth rinses has been proposed as adjuncts to mechanical oral hygiene regimens and is considered a mean to enhance plaque removal (5, 6). Mouthrinsing was first described as an oral hygiene measure in Chinese medicine in 2700 BC (7).

Cetylpyridinium chloride (CPC) is a cationic quaternary ammonium compound that is a common ingredient in over-the-counter mouth rinses (7–10). Schroeder *et al.* (8) first described the plaque-inhibiting effect of CPC, which exhibits antimicrobial activity against gram-positive bacteria and has a fungicidal effect, particularly on yeast (11, 12). Cetylpyridinium chloride binds to the phosphate groups of lipids in cell walls of bacteria. It penetrates the cell and causes membrane damage (13) that leads to leakage of cell components, disruption of bacterial metabolism, inhibition of cell growth and finally cell death (14–16). Because of its surface-active properties, CPC exerts a prolonged effect in the oral cavity by binding to glycoproteins that cover the teeth and oral mucosa (17). The use of CPC-containing mouth rinses has shown to be safe and does not disturb the balance of the oral microbiota (11). A shift in indigenous bacteria from facultative gram-positive streptococci, in particular, to anaerobic gram-negative anaerobic bacteria does not occur (12).

In a systematic review (18), CPC-containing mouth rinses were shown to provide a modest but significant additional benefit in reducing plaque and gingival inflammation when used as an adjunct to either supervised or unsupervised oral hygiene measures. A recent 4-day *de novo* plaque accumulation model (19) showed that a 0.05% CPC rinse was able to reduce plaque formation. In another 3-day crossover '*de novo*' plaque accumulation model (20), 0.07% CPC was found to be more effective than a placebo rinse. According to the guidelines of the American Dental Association (ADA) (21), long-term studies are needed to make claims concerning the effect on gingivitis. The purpose of the present study was therefore to evaluate, over a 6-month period, the effectiveness of a 0.07% CPC mouth rinse with respect to inhibition of plaque formation and gingival inflammation compared to a vehicle control (VC) mouth rinse and to monitor possible shifts in the composition of the microbiota, adverse effects and tooth staining.

Materials and methods

Ethics

The study followed instructions based on the Helsinki principles. The protocol was approved by the Medical Ethics Committee of the Academic Medical Centre (AMC) of Amsterdam under registration number MEC 09 / 098 no. 09.17.0873 and registered at the Dutch Trial Register (NTR1855). The study was scheduled and executed from June to December 2009 at the department of periodontology at the Academic Center for Dentistry Amsterdam, the Netherlands, with a minimum of 60 participants. Recruitment of the participants was performed by e-mail and flyers. Before enrolment, further detailed information was provided at the screening visit by the investigator. The voluntary participants were requested to give their written informed consent, asked to fill out a medical questionnaire prior the start of the study and verified for willingness to comply with the objectives of the study.

Participants

In total, 81 systemically healthy participants were recruited being non-dental students from universities and colleges in and near Amsterdam. Inclusion criteria were ≥18 years of age with at least 20 teeth (minimum of five evaluable teeth per quadrant) and moderate gingivitis with ≥40% bleeding on marginal probing (BOMP) (22, 23). Exclusion criteria were open caries, pockets of 4–5 mm in combination with gingival recession or pockets of ≥6 mm as assessed according the Dutch Periodontal Screening Index (DPSI) scores 3^+ and 4 (24, 25). In addition, orthodontic appliances or removable (partial) dentures, a history of allergic reaction to erythrosine and/or CPC, use of antibiotics in the preceding 3 months, pregnancy and any adverse medical history or long-term medication might interfere with the response variables. In addition, the eligible participants did not use a mouth rinse as part of their daily oral hygiene procedure.

Study design

This was a 6-month, randomized, parallel, double-blinded, placebo-controlled study (see Fig. 1). At baseline, participants were assessed for microbiological and clinical parameters. Subsequently, the dentition was stained for plaque with a suitable dye, for example 0.5% erythrosine disclosing solution (ACTA, Amsterdam, The Netherlands), and the participants received professional oral prophylaxis for a maximum of 30 min performed by experienced dental hygienists. Teeth were scaled and polished to be free of plaque, stain and calculus in order to give the participants an identical start as described by Slot *et al.* (26).

All teeth in two randomly selected contra-lateral quadrants (one upper and one lower quadrant) were clinically examined except for the third molars (27). Randomization for group and quadrant selection was performed using true random numbers, which were generated by sampling and by processing a source of entropy outside the computer. The source was atmospheric noise, which was sampled and fed into a computer without any buffering mechanisms in the operating system (www.random.org). Allocation concealment was accomplished using the sequentially numbered, opaque, sealed envelopes (SNOSE) method (28). The opposing contra-lateral quadrants were used for microbiological sampling. Mouth rinses were identically packed and could only be identified by corresponding subject numbers. Subsequently, every subject received a unique trial number and was randomly assigned to either the CPC group or VC group.

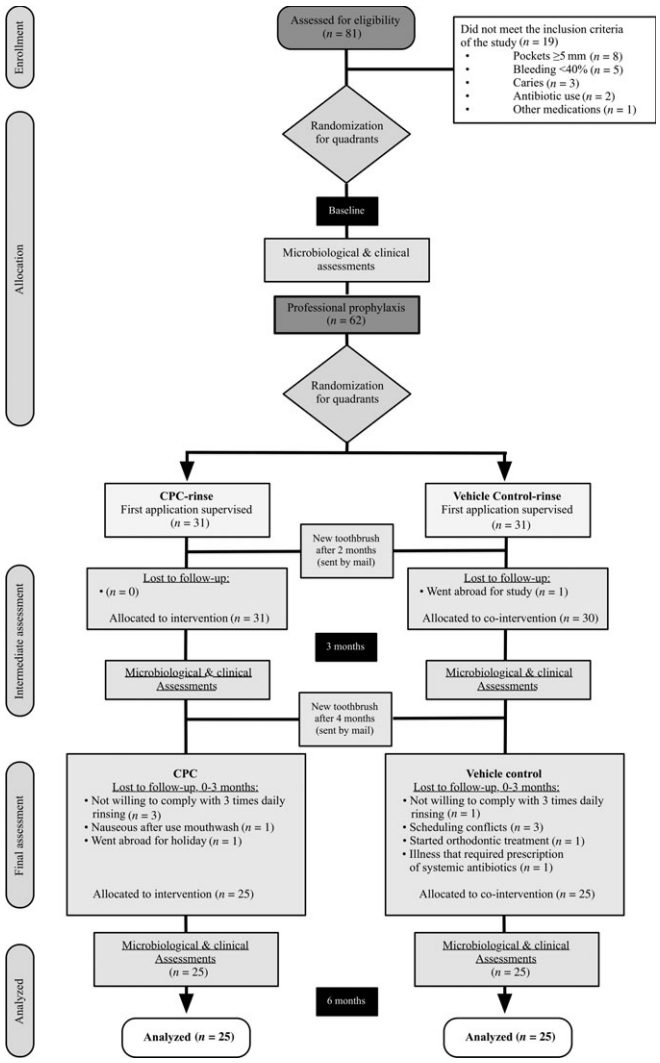

Fig. 1. Flowchart depicting subject enrolment and measurements.

Study products and regimen

The test product was an experimental 0.07% CPC mouth rinse, and the VC mouth rinse was identical to the test product, however, without the 0.07% cetylpyridinium chloride. The bioavailability of this 0.07% CPC product, according to disc retention assay, was approximately 100% (29, 30). The test and control rinses were identically packed by Dentaid (Cerdanyola, Barcelona, Spain), with the same colour and could only be identified by the corresponding participant numbers. The randomization key was held by the principal investigator and the sponsor and was not available to the participants and the examiner. All participants received their assigned products immediately after the professional prophylaxis as well as a demonstration and verbal instruction by the study coordinator (CEB). The participants were then asked to rinse under supervision for the first time with their allocated product. In addition, detailed instruction form was provided that explained how to use of the products. Participants first brushed with a standard toothbrush (VITIS Encias, Dentaid®) and one brush length of dentifrice [Aquafresh (GlaxoSmithKline, Zeist, The Netherlands) containing sodium fluoride without additional chemical plaque inhibitors]. Furthermore, participants were instructed to brush three times daily for 2 min after followed by rinsing with their assigned mouth rinse (15 ml) for 30 s with as recommended by the manufacturer after breakfast, after lunch and before bedtime. Mouth rinse, toothbrushes and dentifrice were supplied throughout the study, to last up till the next appointment. To check for compliance, all bottles were weighed before the products were distributed to the participants; they were re-weighed when they were returned.

Clinical parameters

After baseline measurements, participants returned after 3 and 6 months. Subjects were instructed to brush between 2 and 3 h prior to each appointment to avoid the risk of increased bleeding as a result of tooth brushing (31, 32). All partial mouth examinations were performed in two randomly chosen contra-lateral quadrants (27). The same experienced examiner (PAV) recorded scores using the same conditions, in the following order. As the primary outcome variable, gingival condition was assessed at 6 sites around the selected teeth* by scoring BOMP on a scale of 0–2 (22, 23). As the secondary outcome, plaque was assessed at six sites after disclosing with (Mira-2-Ton®; Hager & Werken GmbH & Co. KG., Duisburg, Germany) and based on a modified Quigley & Hein (33) plaque index as described by Paraskevas *et al.* (34) on a scale of 0–5. Tooth stain was scored for all selected teeth at four sites from the buccal aspect according to the Gründemann Modification of the Stain Index (GMSI) on a scale of 0–3 (35, 36).

Microbiological parameters

To monitor the composition of supragingival plaque during the experimental period, qualitative and quantitative analyses of the dental plaque were performed. As suggested by Heijnsbroek *et al.* (37), before the clinical assessments, at 3 months and at 6 months, supragingival plaque was collected from the buccal sites of the first and second (pre)molars from both the upper and lower jaws in contra-lateral quadrants, which were the opposing areas in relation to those used for clinical assessment. Plaque from the preselected sites was dried with compressed air before a sample was obtained using a sterile Teflon Ash (Neos 425/5; KerrHawe, Bioggio, Switzerland). Plaque samples were pooled and transferred to a vial containing 2 ml of sterile reduced transport fluid (RTF) (38). In addition, a 1-ml sample of unstimulated saliva was obtained and mixed with 1 ml of sterile RTF. All samples were kept at 4°C until transport to the laboratory, where they were vortexed for 60 sec and prepared in 10-fold dilutions in sterile saline. Aliquots of

*Mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual.

100 µl were transferred to selective and non-selective plates, where they were spread equally and incubated. For total aerobic counts, non-selective blood agar plates (Oxoid no. 2, Basingstoke, UK) were incubated at 37°C under 5% CO_2 for 5 days; for total anaerobic counts, non-selective blood agar plates (Oxoid) supplemented with hemin (5 mg l^{-1}) and menadione (1 mg l^{-1}) were incubated at 37°C under 80% N_2, 10% CO_2 and 10% H_2 in the presence of regenerated palladium catalyst for up to 14 days. Selective plates for total streptococci (Mitis salivarius aga; BBL, Cockeysville, MD, USA) and lactobacilli species (Rogosa agar; Oxoid) were incubated anaerobically for 5 days. *Candida* spp. was isolated on Sabouraud agar (BBL). Confirmation of the identity of streptococci, lactobacilli and *Candida* spp. was performed using Gram staining.

Questionnaire

At the 6-month assessment, after completion of the microbiological and clinical assessments, all participants were asked to complete a questionnaire designed to evaluate their attitudes towards the assigned mouth rinses. To assess these subjective items, each participant marked the severity of each symptom on a 10-cm-long visual analogue scale (VAS) with the negative and the positive on the left and right.

Sample size

The American Dental Association (ADA) Acceptance Program Guidelines: Chemotherapeutic Products for Control of Gingivitis (21) does not propose a minimum number of participants, but just states that a sufficient number of participants should be enroled. Therefore, the sample size was calculated *a priori* [PS: Power and Sample Size program (39)] based on a pooled standard deviation (σ) of 0.3 [as taken from gingivitis scores in a previous 6-month mouth rinse study by Paraskevas *et al.* (40)], as well as a detectable difference (δ) of 0.25 (between groups) with an $\alpha = 0.05$ to obtain 80% power. This calculation indicated that 24 subjects in each group would be sufficient. The study was initiated with 31 participants in each group to allow for dropouts.

Data analysis

For each participant, the mean values for each group were calculated. Subject-based data of the CPC and VC groups were compared with regard to plaque and bleeding indices using a univariate analysis, with measurements at 6 months as dependent variables and baseline scores as covariate (41). For post-test and explorative analysis, nonparametric tests were used. The Wilcoxon test was used to test for differences within each regimen over time, whereas the Mann–Whitney *U*-test was used for evaluation between regimens. For GMSI, overall scores were tested using Kruskal–Wallis tests to compare scores between regimens at each assessment. A *t*-test was used to evaluate the VAS scores of the questionnaire data. Chi-squared and binomial tests were used for questions concerning binomial choices. Values of $P < 0.05$ were defined as statistically significant. Microbiological outcomes were compared between groups using a Friedman test (nonparametric repeated-measures analysis).

Based on total colony-forming units (CFU) values for anaerobic and aerobic micro-organisms, a ratio was calculated with total anaerobic CFU in the denominator to indicate the proportion of aerobic bacteria. Reduction of anaerobes was considered a beneficial change. All data were analysed 'intention-to-treat'. It involved data of all randomly assigned participants who provided a full data set (42).

Results

In total, 81 participants were screened, of which 62 participants were enrolled into the study. Twelve participants did not complete the 6-month protocol for various reasons (for further details, see Fig. 1). Baseline demographics were comparable, as shown in Table 1.

Clinical results

For plaque, the mean baseline scores were 1.58 for the CPC group and 1.77 for the VC group ($P = 0.082$). At 3 months, the scores were 1.55 for the CPC group and 1.95 for the VC group ($P = 0.002$). At 6 months, the scores were 1.28 for the CPC group and 1.68 for the VC group ($P = 0.001$). A univariate analysis, with the baseline as the covariate and 6-month scores as dependent variables, showed a difference ($P = 0.002$) between groups in favour of the CPC mouth rinse with respect to plaque scores (Table 2a).

With respect to gingivitis, baseline scores were 1.14 for the CPC group and 1.16 for the VC group ($P = 0.854$). At 6 months, the scores were 1.03 for the CPC group and 1.14 for the VC group ($P = 0.089$). Univariate analysis with the baseline as the covariate and 6-month scores as dependent variables showed no significant differences between groups (Table 2b).

The mean percentage of sites showing staining at baseline was 2.6% for the CPC group and 3.4% for the VC group ($P = 0.958$). With regard to staining, both groups after having received a professional prophylaxis started the study with equally clean teeth. At 3-month staining, this was 10.1% for the CPC group and 1.5% for the VC group ($P = 0.0001$), and at 6 months, it was 13.3% for the CPC group and 2.3% for the

Table 1. **Study subject demographics by group**

	CPC group	VC group	*P*-value
N	25	25	–
Male ♂	5	7	$P = 0.508$*
Female ♀	20	18	
Mean age in years (SD)	22.5 (3.20)	21.1 (2.32)	$P = 0.083$**
Age range	19–30	18–27	–

CPC, cetylpyridinium chloride; VC, vehicle control; SD, standard deviation. *Chi-square analysis. **Independent *t* -test analysis.

Table 2. **(a) Mean (SD) plaque Quigley & Hein scores (Q&H) values and percentage (SD) plaque site for both the CPC (N=25) and VC (N=25) groups at the three assessments. (b) Mean (SD) Bleeding on Marginal Probing (BOMP) values and percentage (SD) bleeding sites for both the CPC and VC groups at the three assessments. (c) Mean (SD) percentages of sites showing staining according to the Gründemann Modification of the Stain Index (GMSI) for both the CPC and VC groups at the three assessments. (d) Description of the Lobene tooth stain intensity scale in percentage (SD) of sites for both the CPC and VC groups at the three assessments**

	Plaque (Q&H)		Baseline	3 months	6 months	*P*-value**
(a)						
	CPC group	Mean	1.58 (0.39)	1.55 (0.45)	1.28 (0.37)	
Overall mean		Percentage	72.2 (12.7)	68.2 (13.8)	63.0 (13.5)	0.002
	VC group	Mean	1.77 (0.40)	1.95 (0.38)	1.68 (0.37)	
		Percentage	78.1 (11.6)	81.1 (10.6)	75.3 (12.1)	
Mean difference			0.19 (0.54)	0.40 (0.41)	0.40 (0.42)	
P-value*			0.082	0.002	0.001	
	Gingivitis (BOMP)		Baseline	3 months	6 months	*P*-value**
(b)						
	CPC group	Mean	1.14 (0.31)	1.20 (0.20)	1.03 (0.26)	
Overall mean		Percentage	64.3 (15.5)	65.9 (9.5)	58.9 (14.2)	0.061
	VC group	Mean	1.16 (0.31)	1.12 (0.23)	1.14 (0.29)	
		Percentage	64.2 (14.0)	62.5 (10.6)	65.6 (14.2)	
Mean difference			0.02 (0.47)	0.08 (0.31)	0.11 (0.39)	
P-value*			0.854	0.281	0.08	
	Staining (GMSI)		Baseline	3 months	6 months	*P*-value**
(c)						
	CPC group	Percentage	2.6% (4)	10.1% (9)	13.3% (8)	
Overall mean						<0.0001
	VC group	Percentage	3.4% (8)	1.5% (2)	2.3% (10)	
Mean difference			0.8% (9)	8.6% (9)	11% (11)	
P-value*			0.958	<0.0001	<0.0001	
	Staining (GMSI) Lobene intensity		Baseline, %	3 months, %	6 months, %	
(d)						
CPC group	Score 0		97.4	89.9	86.7	
	Score 1		2.3	8.1	8.7	
	Score 2		0.3	2.1	4.2	
	Score 3		0	0	0.4	
VC group	Score 0		96.6	98.5	97.7	
	Score 1		2.5	1.5	2.3	
	Score 2		0.9	0	0.1	
	Score 3		0	0	0	

*Mann–Whitney *U*-test used for post-testing, **univariate analyses with mean baseline data as covariate and 6-month data as dependent variables.

VC group ($P = 0.0001$, Table 2c). In case of toothstaining, the intensity of the stain was primarily score I (Table 2d).

Microbiological results

As presented in Table 3, the mean total aerobic and anaerobic counts were affected by the CPC and VC mouth rinses; there was a 2- to 3-fold significant decrease in the total anaerobic count in the CPC group ($P < 0.05$), but not in the VC group at 6 months relative to baseline counts. A significant increase in both the CPC ($P < 0.05$) and VC ($P < 0.001$) groups for total aerobic counts was noted at 3 months, although these differences were not observed at 6 months (Table 3). The ratio between the total cultivable aerobic and anaerobic counts changed in both the CPC and VC participants, although at 3 months the increase was more pronounced in the CPC group (5.3 compared to 3.4). At 6 months, both ratios were comparable and still higher (4.3) than baseline values. Mean levels of total streptococci did not change during the 6-month period in the CPC group. In the VC group, a slight increase in total streptococci was observed at 3 months and at 6 months relative to the baseline counts. Among the VC participants,

Table 3. **Microbiological data and statistical analysis with respect to total CFU per ml (SD) and streptococci, lactobacilli and Candida in particular and prevalence at baseline, 3 and 6 months derived from supragingival plaque and saliva**

	Baseline		3-months		6-months		
	Prevalence (%)	Cells/mL (SD)	Prevalence (%)	Cells/mL (SD)	Prevalence (%)	Cells/mL (SD)	*P* value*
Total cfu $Ø_2$							
CPC group	100	4.7×10^8 (8.4×10^8)	100	3.6×10^8 (2.9×10^8)	100	1.5×10^8 (1.4×10^8)	<0.05
VC group	100	3.0×10^8 (2.8×10^8)	100	6.8×10^8 (8.1×10^8)	100	1.6×10^8 (1.7×10^8)	ns
Total cfu O_2							
CPC group	100	9.0×10^8 (1.4×10^9)	100	1.9×10^9 (1.9×10^9)	100	6.5×10^8 (7.3×10^8)	ns
VC group	100	5.7×10^8 (6.0×10^8)	100	2.3×10^9 (1.8×10^9)	100	6.9×10^8 (6.2×10^8)	ns
Ratio CPC $O_2/Ø_2$		1.9		5.3		4.3	
Ratio VC $O_2/Ø_2$		1.9		3.4		4.3	
Streptococci (cfu)							
CPC group	100	1.2×10^7(1.6×10^7)	100	2.4×10^7 (2.9×10^7)	100	4.4×10^7 (6.8×10^7)	ns
VC group	100	9.9×10^6(1.7×10^7)	100	4.1×10^7 (6.3×10^7)	100	6.5×10^7 (8.7×10^7)	<0.01
Lactobacilli (cfu)							
CPC group	76	6.9×10^2(1.4×10^3)	64	7.3×10^3 (1.2×10^4)	60	2.1×10^4 (5.0×10^4)	ns
VC group	76	3.4×10^4 (8.2×10^4)	64	1.2×10^4 (2.4×10^4)	60	4.2×10^3 (1.3×10^4)	ns
Candida							
CPC group	68	8.8×10^3 (1.8×10^4)	48	9.0×10^3 (1.1×10^4)	52	6.0×10^3 (8.4×10^3)	<0.05
VC group	68	8.2×10^3 (1.2×10^4)	40	2.1×10^4 (1.7×10^4)	40	1.8×10^4 (1.5×10^4)	ns
Saliva							
CPC group	76	1.1×10^3 (1.6×10^3)	60	4.7×10^2 (5.5×10^2)	56	3.7×10^2 (3.0×10^2)	ns
VC group	68	1.0×10^3 (1.5×10^3)	64	3.8×10^2 (5.3×10^2)	52	4.2×10^2 (6.7×10^2)	ns

CPC, cetylpyridinium chloride (n = 25); VC, vehicle control (n = 25); $Ø_2$, anaerobic; O_2, aerobic; SD, standard deviation; ns, not significant.
*Friedman test (nonparametric repeated-measures analysis) baseline-6 months.

Table 4. **Mean amount of mouth rinse product used per participant in ml**

	Baseline–3 months	3–6 months	*P*-value*
CPC group (n = 25)	3210	3540	0.043
VC group (n = 25)	3390	3520	0.243
P-value**	0.312	0.879	

*paired samples t-test; **independent t-test.

mean levels of lactobacilli decreased during the experimental period within ± 1 log, whereas the prevalence of lactobacilli-positive participants decreased from 76% at baseline to 60% at 6 months. No significant changes in lactobacilli counts were observed in the CPC group during the 6-month period. The number of participants positive for *Candida* spp. decreased in both groups, whereas absolute counts in culture-positive participants decreased slightly in the CPC group ($P < 0.05$). No significant changes in *Candida* spp. counts were noted in the saliva from both groups.

Participant attitudes and adverse events

The amount of used mouth rinse was calculated per participant. No significant differences were observed between the CPC and VC groups regarding the amount of mouth rinse used during the first or the second part of the study. However, an analysis of these data revealed that participants' had used less than the prescribed amount of mouth rinse over the 6-month period. Table 4 provides additional details.

Table 5 presents the data with respect to the questionnaire, which was completed by the participants after their 6-month appointment. No significant differences were observed concerning any of the addressed items.

After visit 1, two participants reported staining as an adverse event. After completion of the study when the product allocation was revealed, these participants were shown to have used the CPC rinse. One subject in the CPC group complained about nausea and discontinued participation in the study (43).

Discussion

In the present study, rinsing with the CPC mouth rinse, three times daily, significantly reduced the level of dental plaque scores (by approximately 24%) relative to the VC product at 3 and 6 months. With respect to gingivitis, no significant difference was found between groups at 6 months. However, the overall analysis correcting for baseline scores revealed a trend towards a significant effect in favour of the CPC group ($P = 0.061$). The magnitude of this effect is limited (0.11 on a 2-point bleeding score). To be clinically important, a substantial change in outcome would be needed. With regard to the negative side effect of staining, a significant difference was

Table 5. **Questionnaire responses for the visual analogue scale (scored from 0 to 10)**
The mean scores are presented for the CPC group and VC group

	Extremes		Mean scores (SD)		
Paraphrase	From	To	CPC group (n = 25)	VC group (n = 25)	P-value*
Sensitive mucosa and/or teeth	Not at all	Very much	2.79 (2.64)	2.60 (2.51)	0.798
Burning sensation	Not at all	Very much	2.71 (2.69)	3.00 (2.83)	0.710
Experience dry mouth	Not at all	Very much	2.16 (2.37)	2.88 (2.81)	0.328
Experience numbness	Not at all	Very much	1.53 (2.00)	1.54 (2.31)	0.984
Staining of teeth	Not at all	Very much	1.58 (1.99)	2.67 (3.17)	0.153
Taste perception	Very bad	Very good	6.70 (1.44)	5.88 (1.84)	0.084
Duration of taste	Very short	Very long	5.38 (1.99)	5.60 (1.73)	0.667
Opinion regarding rinsing time	Very short	Very long	5.16 (1.51)	5.03 (1.74)	0.776
Alteration of taste	Negatively changed	Positively changed	4.74 (0.81)	4.29 (0.80)	0.058

*independent t-test.

observed at 3 and 6 months, with the CPC group displaying more staining, although patients did not complain of this in the questionnaires.

Other studies

These results are similar to those of previous studies. Versteeg *et al.* (20) showed that the 0.07% CPC mouth rinse, which was identical to the present test product, was capable of reducing plaque formation by approximately 47%. Recently, Costa *et al.* (44) showed a clear beneficial effect of the adjunctive use of the experimental 0.07% mouth rinse when compared with a placebo. Garcia *et al.* (19) tested a lower-concentration 0.05% CPC mouth rinse and found 25% plaque inhibition in a *de novo* plaque formation model. However, Rioboo *et al.* (45) evaluated a 0.05% CPC mouth rinse over a 4-week study and failed to establish a difference between the test and control products with respect to gingivitis, although they reported a trend for differences in plaque scores. Haps *et al.* (18) systematically evaluated the effects of CPC-containing mouth rinses when used as adjuncts to either supervised or unsupervised oral hygiene regimens in a systematic review (SR) and showed, based on a meta-analysis, a small but significant additional benefit of CPC in reduction of plaque and gingival index scores.

Compliance

Compliance in the present study was measured by the average amount of mouth rinse used during the 6-month period for both groups. No significant differences between the CPC and VC groups were observed with respect to the amount of mouth rinse used during either the first or second part of the study. Presumably, the participants rinsed 3 times daily only for 50% of the study duration. On the other occasions, participants may have possibly rinsed only twice daily. The cause of this lack of compliance may be the inconvenience associated with for instance bringing the toothbrush, dentifrice and bottle of mouth rinse with them to work for the afternoon oral hygiene procedures. This lack of compliance was also shown in two other studies (46, 47), which noted that only 30 to 50 per cent of patients were highly compliant with the suggested oral hygiene procedures up to a period as short as 30 days after receiving instructions. Obviously, if patient compliance is lacking, effects of a daily antimicrobial rinse regimen will be suboptimal (48). The practicability of a mouth rinse should therefore match with a person's long-term compliance, otherwise the value of such a mouth rinse is negligible.

Bleeding scores

The non-significant trend on gingival bleeding scores in the present study is not in support of a recent systematic review (18), which showed a significant effect of CPC on gingivitis. The reason for this is unclear. The higher CPC concentration found in two (49, 50) of the included experiments in the meta-analysis of the SR (18) used a higher concentration (0.1%) which may have contributed to the enhanced effect, although the participants only rinsed once a day. This resulted in these two studies in a total delivery of 15 mg CPC per day as compared to the intended 31 mg in the present study. Other factors that may explain the differences among the present study and the outcome of the SR are differences in formulations (e.g. presence or absence of alcohol) or the lack of compliance to the three times daily usage (Table 4). Also, as suggested by Addy *et al.* (51), studies attempting to assess the effect of mouth rinses on plaque formation are hampered not only by the number of components in the formulation but also by the mechanical action of the toothbrush. Additionally, varying compliance may have resulted in different outcomes. In general, the results from this study show that CPC rinsing has a clear tendency towards an effect on gingivitis, but this effect is small. This will need a larger study population to provide significance. In addition, the present study only compered the CPC formulation to a vehicle control. When designing another study, a group using a positive or benchmark control should be considered. The present study used clinical surrogate outcome measurements being plaque, bleeding and staining scores. And therefore it is impossible to draw conclusions based on hard-outcomes like tooth loss. The question how many more teeth

will be maintained, if patients use the rinsing solution three times a day for many years, remains unclear. However this was not the aim of the current study and will need a different methodological approach when a study will be designed for answering this question.

Bioavailability

The FDA subcommittee states that CPC bioavailability is indicative of a product's performance as '*it readily defines the amount of drug available for deposition at the site of action*' (52). Consequently, the FDA subcommittee recommends CPC bioavailability ranging from 72% to 77%. However, the bioavailability of most CPC formulations has not been properly reported (19). It has been shown that a possible interaction between the active agents and the excipients within the formulation can influence CPC bioavailability in a specific product. When used immediately after brushing with toothpaste, the activity of the mouth rinse could be inhibited by the toothpaste formulation (11, 53). Because the positively charged hydrophilic region of CPC is critical for its antimicrobial activity, mouth rinse formulations should not contain ingredients that diminish or compete with the activity of this cationic group. When the formulation is improperly prepared, inactivation of CPC is likely to occur as a result of chemical reactions such as complexing, micelle formation or other sources of deactivation. Therefore, is it recommended that the bioavailability of CPC in each formulation should be determined to minimize such a possibility (43). For the present study as in a previous study (20), a CPC rinse with approximately 100% bioavailability (according to Dentaid International, Barcelona, Spain) was used.

Safety and adverse effects

The safety of CPC has been extensively evaluated and confirmed, based on data collected from animal and pharmacokinetic studies, via assessment of adverse events in randomized, *placebo*-controlled clinical trials (54–58) and from post-market spontaneous adverse event data reported to the manufacturer and the FDA. In the present study, the participants reported no serious adverse effects, and there was no difference in taste perception between the CPC rinse and its true *placebo*. Compared to the VC, the CPC rinse resulted in a clinically small (10.4%) but significant increase in tooth stain scores (Table 2c); however, this was not an item which came out as a significant difference in the patient perception questionnaire. In fact, patients in the VC group self-reported more tooth staining than patients in the CPC group (Table 5). Staining following the use of CPC mouth rinse is a known side effect according to the systematic review of Haps *et al.* (18).

Microbiological monitoring

The culture technique was used for microbiological analysis in this study to provide an open test system that enables the determination of total aerobic and anaerobic bacterial counts. To compare potential changes in oral microbiota, microbiological parameters were established at baseline before SRP. Subsequently, microbiological assessments were performed at 3 months and after termination of the test period at 6 months.

During the experimental period of 6 months, no negative shifts in microbiota were observed in the dental plaque or saliva. An interesting observation was the increase in the ratio of total aerobic to total anaerobic counts, which occurred for both the CPC mouth rinse and VC mouth rinse. However, this shift was most pronounced in the CPC group at 3 months. Clinically, this is a relevant environmental shift towards a more beneficial microbiota. However, this could be also be due to the SRP preformed after baseline measurements. Still, the CPC seems to have an additional effect during the first 3 months (ratio aerobe/anaerobe, Table 3). This parameter can be interpreted as a determinant of improved plaque quality (59). According to the regulations of the ADA Acceptance Program Guidelines: Chemotherapeutic Products for Control of Gingivitis (21), products should be evaluated for both clinical and microbiological parameters. The requirements include qualitative microbial plaque improvement and safety of the product in terms of emergence of opportunistic pathogens. The oral microbiota should be monitored in participants during the study for the development of opportunistic and pathogenic organisms. Evidence must be provided that the oral microbiota has not been adversely affected. Which organisms should be monitored in relation to the safety of a novel mouth rinse product is not specifically defined, however. For this study, *Candida* spp. were selected as indicators of potential overgrowth arising from bacterial inhibition by CPC.

A decrease was observed in the number of *Candida*-positive plaque samples among the CPC participants whereby the difference in the *Candida*-positive plaque between baseline and 6 months was statically significant ($P < 0.05$), and no candida overgrowth occurred. The decrease in the amounts of candida is interesting, and it might also be clinically relevant. Patients suffering of recurrent candida infections may benefit from CPC mouth rinse, as alternative for prophylactic candida medications. This could be a topic for further research. On the basis of these observations, no apparent changes in microbiology occurred that would indicate increased risk for opportunistic infections. This is in agreement with a 6-month study on the microbiological effects of CPC (0.07%), which showed that the subgingival microbiota was not significantly affected (60).

A limitation of the performed study is that microbiota and clinical assessments were obtained from contra-lateral quadrants in order not to affect the clinical assessment of plaque by removing it before sampling Therefore, the microbiological data might not correlate directly with the clinical data. However, a study performed by (27) showed that partial assessments (half-mouth) were similar in magnitude to those derived from full-mouth examinations. Therefore, it seems legitimate to obtain assessments from different sites with the intention not to influence proper sampling and scoring.

Conclusions

The results of this clinical trial showed that the use of a 0.07% CPC mouth rinse was significantly more effective in reducing plaque scores than the use of the VC product. No significant differences between the CPC and the VC groups with respect to bleeding scores were observed at 6 months. The test product was well accepted and did not cause any serious adverse clinical side effects or negatively affected the oral microbiota.

Clinical relevance

Scientific rationale for the study

CPC-containing mouth rinses were shown to provide a modest but significant additional benefit in reducing plaque and gingival inflammation. To assess the effect on parameters of gingivitis, long-term studies were needed. Therefore, the 0.07% CPC mouth rinse was evaluated over a 6-month period.

Principle findings

The 0.07% CPC mouth rinse was significantly more effective in reducing plaque scores than the use of the vehicle control. No serious clinical side effect of the CPC mouth rinse was reported nor it changed the oral microbiota composition.

Practical implications

Rinsing twice daily with a 0.07% CPC mouth rinse delivers therapeutic benefits by inhibiting plaque accumulation. Thereby it decreased the amount of candida in the CPC group, which can be considered as clinically relevance. Patients suffering of recurrent candida infections may benefit from a CPC mouth rinse, as alternative for prophylactic candida medications.

Limitation

Due to the methodological approach and chosen surrogate outcome parameters of the present study, it was impossible to draw conclusions based on hard outcomes like tooth loss.

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