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The plaque inhibitory effect of a CPC mouthrinse in a 3-day plaque accumulation model – a cross-over study

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Versteeg PA, Rosema NAM, Hoenderdos NL, Slot DE, Van der Weijden GA. The plaque inhibitory effect of a CPC mouthrinse in a 3-day plaque accumulation model – a cross-over study.

Abstract: *Objective:* To test the plaque inhibitory effect of an experimental 0.07% cetylpyridinium chloride (CPC) mouthrinse in a 3-day plaque accumulation model in a cross-over design. *Material and Methods:* A total of 30 subjects (non-dental students), ≥18 years of age, were randomly assigned to use one of three different mouthrinses three times a day. After 3 days, the subjects returned for the clinical assessments and received a questionnaire to evaluate their attitude towards the product used by them. The cross-over procedure was repeated twice to have all subjects use all products. *Results:* A total of 29 subjects completed the protocol and this resulted in a statistically significant difference between the three groups ($P < 0.001$) with regard to plaque scores. Over three sessions, the mean plaque scores were 2.17 for the control product, 1.14 for the CPC group and 1.12 for the 0.1% Hexetidine product (positive control). Results of the questionnaire show that, compared with hexetidine, the taste of the CPC was appreciated better, and less oral sensations were observed following rinsing. *Conclusion:* The CPC mouthrinse proved to be effective in inhibiting 'de novo' plaque formation to an extent similar to that of a 0.1% hexetidine product. Compared with hexetidine, the taste of the CPC was appreciated better and less oral sensations were observed following rinsing.

Key words: cetylpyridinium chloride; clinical trial; dental plaque; hexetidine; mouthrinse

Introduction

Dental plaque is a biofilm that forms naturally on the surfaces of exposed teeth (1). It is a complex organized microbial

community which is the primary aetiological factor for the most frequently occurring oral diseases, such as dental caries and periodontal diseases. Although the dental biofilm cannot be eliminated, it can be controlled with comprehensive mechanical and oral hygiene practices. Routine toothbrushing is widely recognized as the first step to mitigate the effects of dental plaque and maintain oral health (2). However, certain patients may not be willing or able to perform adequate mechanical plaque removal on a regular basis. These patients could benefit from chemotherapeutic anti-plaque agents as adjuncts to mechanical removal. Topical antimicrobials in dental products have four general mechanisms of action. They can decrease the rate of new plaque accumulation, decrease or remove existing plaque, suppress the growth of pathogenic microflora or inhibit the production of virulence factors (3).

Today, therapeutic ingredients available in mouthrinses include various metal ions such as stannous, zinc, copper and also essential oil mixtures, chlorhexidine (CHX) and cetylpyridinium chloride (CPC) (4–6). Most of these antimicrobial ingredients derive their effects from co-solubilization of the active ingredient into the hydrophobic portions of the bacterial cell walls (7–9).

Hexetidine belongs to the group of pyrimidine derivatives. After discovering the specific antibacterial and fungicidal effect of hexetidine, many clinical studies verified the sensitivity of bacteria against hexetidine (10, 11). Formulated as a mouthrinse, it is available in a number of markets worldwide, with indications for the treatment of a variety of conditions of the oropharynx. Early, mainly open-label, studies on hexetidine mouthrinse indicated positive benefits when used in the treatment and prevention of gingivitis (12, 13). Recent studies have shown, in subjects who refrained from other oral hygiene measures, favourable effects on plaque and gingivitis (14, 15) and less tendency for stain production when compared with 0.1% CHX (15).

The quaternary ammonium compound CPC is a cationic surface-active agent and has some similarities to CHX in this respect. CPC has a broad antimicrobial spectrum with a rapid bactericidal effect on Gram-positive pathogens and a fungicidal effect on yeast in particular (16). It is assumed that interaction with bacteria occurs by causing disturbance of the membrane function, leakage of cytoplasmic material and ultimately the collapse of the intra-cellular equilibrium (16, 17). A plaque-inhibiting effect caused by CPC was first described by Schroeder & Hirzel (18). In 2003, the Food and Drug Administration (FDA) Plaque Subcommittee classified CPC as safe and efficacious for the treatment of plaque-induced gingivitis, when formulated in a mouthrinse within a concentration range of 0.045–0.10 (19). CPC is perhaps the most common ingredient in over-the-counter mouthrinses and is usually found at a concentration of 0.05% (20–22). Research has demonstrated that CPC mouthrinses have anti-plaque activity when used alone and in conjunction with toothbrushing (23–28). Recently, the meta-analysis from a systematic review fully supported the plaque- and gingivitis-inhibiting effect of CPC containing mouthrinses (29).

However, it is important to note that all CPC formulations do not necessarily provide the same type or magnitude of benefits. Product formulation has a significant impact on the bioavailability of CPC. Formulations with high bio-available CPC are associated with greater biological activity and therefore suggest an increased probability for clinical efficiency. Rinses with lower CPC concentrations or with less chemically available CPC are marketed as cosmetic products for the temporary control of halitosis, whereas a high bio-available CPC formulation delivers gingival health benefits (30). The efficacy of CPC can be increased by doubling the frequency of rinsing (31). The substantivity of CPC appears to be only 3–5 h (32).

This study aimed to test whether a newly formulated 0.07% CPC mouthrinse with approximately 100% bioavailability (DentAid International, Barcelona, Spain) has the potential to inhibit 'de novo' plaque formation compared with a placebo and a hexetidine mouthrinse.

Material and methods

Study population

A total of 30 subjects (non-dental students) were recruited after screening to take part in the study. The investigator provided all subjects detailed information about the study, first in a recruitment letter and second at the screening visit. They received a written explanation of the background of the study, its objectives and their involvement. Before screening for their suitability, the subjects were requested to give their written informed consent. The subjects were required to fulfil the following criteria: ≥18 years of age; a minimum of five evaluable teeth in each quadrant (with no partial dentures, orthodontic banding or wires) and absence of oral lesions and/or periodontal pockets >5 mm, the absence of pregnancy, systemic diseases such as diabetes and any adverse medical history or long-term medication. In addition, subjects allergic to any of the mouthrinse components were excluded from the study. The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and are consistent with good clinical practice. The trial coordinator was responsible for allocation concealment. Medical ethics

approval (MEC 08/112) was obtained prior to the start of the study. The study has also been registered by the Dutch Trial Register (NTR1329).

Study design

The study involved a 3-day 'non-brushing' experiment and had a randomized, double-blind, 3-arm cross-over design. All participants received a professional oral prophylaxis performed by experienced dental hygienists. The teeth were scaled and polished with the purpose of making them free from plaque, stain and calculus. After debridement, a disclosing solution was applied with a cotton swab. All remaining visible plaque was removed. Subsequently, special attention was given to interproximal areas. Unwaxed floss was used for professional interdental cleaning. Distal of the last molars bandage tape (Cotton Tamponning Bandage 1 × 5 m sterile Hartmann®, Heidenheim, Germany) was used to make sure that all remnants were removed. To assure that all deposits were removed, a second disclosing episode was carried out after which all remaining visible plaques were removed. Subjects received a unique trial number and were randomly assigned to one of the six crossover treatment sequences as described by Newcombe *et al*. (33). The test products were a control placebo mouthrinse with no active ingredients (C-negative control), an experimental 0.07% CPC mouthrinse (CPC-test product) and a 0.1% hexetidine mouthrinse (HEX-positive control). The bioavailability of this 0.07% CPC product according to Disk Retention Assay is approximately 100% (30, 34). All products, placebo mouthrinse (DentAid, Barcelona, Spain), CPC mouthrinse (DentAid, Barcelona, Spain) and Hextril® (Johnson & Johnson Consumer BV, Almere, The Netherlands), were identically packed and could only be identified by corresponding subject numbers. Randomization was performed using random numbers generated by atmospheric noise (http://www.random.org). All subjects were instructed to use 15 ml of rinsing solution for 30 s at each occasion. They completed their first rinsing in the presence of the study investigator with their allocated product. The subjects received a timer with alarm to keep track of the assigned rinsing time. Rinsing, drinking or eating was not allowed for 30 min after each rinsing procedure. The allocation of products was carried out by a third person not directly involved in the research project (PAV). All subjects were instructed to use their allocated products three times a day. Once in the morning after breakfast, once in the afternoon after lunch and once at night, before they went to sleep. All participants were instructed to refrain from using any other oral hygiene measures. To check for compliance, the subjects were asked to register the time of use of the products onto a calendar record chart. After 3 days, the subjects returned to the clinic for the clinical assessments. The day prior to each appointment, all subjects received an SMS message as a reminder.

First, gingivitis was assessed and subsequently the plaque was scored. All measurements were carried out under the same conditions by two investigators who were blinded to product allocation (Plaque Index: NAMR, Bleeding Index: NLH). After the clinical assessment, all subjects received a questionnaire (Table 1) to evaluate their attitudes with regard to the product used, using a visual analogue scale. For each of the questions, the subjects marked a point on a 10-cm-long uncalibrated line with the negative extreme response (0) at the left end and the positive extreme (10) at the right end. At the end of each treatment period of the cross-over design, test products were collected and all subjects entered a 10-day washout phase to minimize carry-over effects and habitual oral hygiene procedures were resumed. Any adverse events reported by the

Table 1. **Complete questions from VAS score (from 0 to 10)**

		With extremes	
Paraphrase	Complete question	From	To
Taste perception	How was the taste of the product?	Very bad	Very good
Duration of taste	How long did the taste remain?	Very short	Very long
Alteration of taste	How was your taste of food and drinks affected?	Negative change	Positive change
Sensitivity	Did you experience sensitivity in your mouth and/or the teeth because of the mouthwash?	Not at all	Very much
Burning sensation	Did you experience a burning sensation in the mouth because of the mouthwash?	Not at all	Very much
Dry mouth	Did you experience a dry mouth because of the mouthwash?	Not at all	Very much
Numbness feeling	Did you experience a numbness feeling in the mouth because of the mouthwash?	Not at all	Very much
Staining	Did you experience staining on the teeth because of the mouthwash?	Not at all	Very much
Cleanliness	Did you have the feeling that your teeth were clean for the last 3 days?	Not at all	Very much

subjects during the course of the study were appropriately recorded.

Clinical assessments

Gingivitis was assessed using the Bleeding on Marginal Probing index as described by Van der Weijden *et al.* (35) and Lie *et al.* (36). The gingival margin was probed at an angle of approximately 60° to the longitudinal axis of the tooth and the absence or presence of bleeding was scored within 30 s of probing on a scale of 0–2 (0 = non-bleeding, 1 = pin-prick bleeding and 2 = excess bleeding).

Plaque was assessed using the modified Quigley & Hein Plaque Index (PI) as described in detail by Paraskevas *et al.* (37), where the absence or presence of plaque was recorded on a 6-point scale (0–5). Plaque was disclosed using a new cotton swab with fresh disclosing solution (Mira-2-Ton®; Hager & Werken GmbH & Co. KG, Duisburg, Germany) for each quadrant. Six surfaces per tooth were examined for both parameters (disto-buccal, mid-buccal, mesio-buccal, disto-lingual, mid-lingual and mesio-lingual).

Data analysis

The sample size of 30 was calculated *a priori* in such a way that with an alpha of 0.05, a difference of 0.24 (between groups) of the PI could be identified with 80% power, based on a pooled SD of 0.45 as derived from previous studies. The plaque scores were used as the main response variable. All analyses comparing differences between the test and control groups were performed using nonparametric tests. Explorative analyses were performed to investigate the origin of the overall differences. Data were categorized according to upper and lower jaw, and tooth types and surfaces. Data obtained from the questionnaire were analysed using parametric tests. Statistical analyses evaluating the efficacy of the products were carried out irrespective of the product allocation of the groups. Values of $P < 0.05$ were accepted as statistically significant.

Results

Of the 30 subjects (9♂, 21♀ aged 18–45 years) who started, 29 completed the study and were deemed evaluable for analyses. One subject (♀) chose not to continue the trial after having an adverse event with a parotid swelling after rinsing with the HEX product for four times (38).

Results of the questionnaire indicate that the questions concerning discolouration showed no statistically significant difference. On average, subjects expressed negative taste observation of the hexetidine product which persisted over a long time and resulted in negative taste alteration of food and drink. Between the CPC group and the HEX group, subjects expressed statistically significant differences in favour of group CPC with respect to sensitivity, numbness, dryness and burning sensation (Table 2).

A statistically significant difference between the three groups ($P < 0.001$) with regard to plaque scores was observed. Over three sessions, the mean plaque scores were 2.17 for the C group, 1.14 for the CPC group and 1.12 for the HEX group. Post-testing showed a statistically significant difference between the C group and the CPC group ($P < 0.001$) and also between the C group and the HEX group ($P < 0.001$). There was no statistically significant difference between the CPC group and the HEX group. With respect to gingivitis, the mean bleeding scores over the three sessions were 0.54 for the C group, 0.54 for the CPC group and 0.53 for the HEX group. No statistically significant differences could be observed between the groups (Table 3).

Table 2. **Results questionnaire**

Group	Mean (SD)			*P*-value			
Question	C	CPC	HEX	C-CPC-HEX†	C-CPC*	C-HEX*	CPC-HEX*
1 Taste	6.75 (1.75)	5.67 (2.15)	2.91 (2.49)	< 0.001	0.048	< 0.001	< 0.001
2 Taste duration	2.88 (1.79)	5.09 (2.52)	8.27 (2.14)	< 0.001	< 0.001	< 0.001	< 0.001
3 Food/drink	4.92 (0.56)	3.84 (2.05)	1.00 (1.45)	< 0.001	0.010	< 0.001	< 0.001
4 Sensitivity	2.37 (2.68)	2.53 (2.46)	4.21 (3.03)	0.021	0.776	0.006	0.006
5 Burning	1.09 (1.18)	2.53 (2.34)	5.78 (2.63)	< 0.001	0.003	< 0.001	< 0.001
6 Dryness	1.81 (1.75)	1.73 (1.72)	4.74 (3.01)	< 0.001	0.883	< 0.001	< 0.001
7 Numbness	0.72 (1.03)	2.11 (2.53)	5.77 (3.05)	< 0.001	0.005	< 0.001	< 0.001
8 Staining	2.08 (2.32)	1.75 (1.87)	2.89 (2.62)	0.158	0.481	0.223	0.038
9 Cleanliness	1.80 (1.70)	5.19 (2.08)	5.98 (2.14)	< 0.001	< 0.001	< 0.001	0.129

*Paired *t*-test.
†Paired ANOVA-test.

Table 3. **Mean (SD) plaque scores and bleeding scores for all groups**

Group	*n*	Plaque	Bleeding
C	29	2.17 (0.46)	0.54 (0.25)
CPC	29	1.14 (0.42)*	0.54 (0.24)
HEX	29	1.12 (0.47)*	0.53 (0.22)
P-value (Friedman)		<0.001	0.941

*Statistically significant different compared with group C $P < 0.001$ (Wilcoxon).
Standard deviation in parentheses.

Discussion

The idea of employing a chemical agent that would act in a manner identical to that of a toothbrush and remove bacteria from the tooth surface is an attractive proposition. Such an agent would be expected to reach all tooth surfaces and thereby be totally effective and safe. For this reason, the idea of chemical plaque removal agents has attracted the terminology of the 'chemical toothbrush'. Despite the ideal nature of the toothpaste vehicle, most chemical plaque-control agents have been evaluated and later formulated in the mouthrinse vehicle. Mouthrinses vary in their constituents but are usually considerably less complex than toothpastes. They can be simple aqueous solutions but the products purchased by the general public need to be stable and acceptable in taste. This usually requires the addition of flavour, colour and preservation additives such as sodium benzoate. Recently, Haps *et al.* (29) reviewed the literature concerning CPC containing mouthrinses as effective adjuncts to toothbrushing in the prevention of plaque accumulation and gingival inflammation. On the basis of the extracted data, the authors' conclusion supports the existing evidence that CPC containing mouthrinses, when used as adjuncts to either supervised or unsupervised oral hygiene, provide a small but significant additional benefit in reducing plaque accumulation and gingival inflammation.

This study evaluates the plaque-inhibiting effect of a 0.07% formulated CPC mouthrinse with a high bioavailability. This was assessed in a 3-day non-brushing model which allows plaque to accumulate freely. This design has been used previously to assess the effect of various mouthrinses (39–45). Simonsson (46) and Zee *et al.* (47) also used this 3-day model to discern between 'rapid' and 'slow' plaque formers. Studies performed by Lang *et al.* (48), Breckx *et al.* (49), Goh *et al.* (50), Quirynen *et al.* (51), Ramberg *et al.* (52, 53), Daly & Highfield (41) and Rudiger *et al.* (54) all confirmed that the periodontal condition is of foremost importance in the rate of de novo plaque formation. Varying levels of gingival health may introduce an unwanted effect. Therefore, in this study, in addition to plaque levels, the level of gingival health was assessed to make sure that this was not an interfering factor with the study outcome.

This study has demonstrated that rinsing with an antiseptic mouthrinse three times a day significantly inhibits plaque. It was decided to rinse three times a day based on the substantivity data for CPC (55), which is somewhere between 180 and 300 min. This is considerably less than the >7 h for 0.2% CHX which is to be regularly used two times a day. One adverse event involving a swollen parotid gland was observed in the hexetidine group. As far as the authors are concerned, this has never been described for this particular product. However, it is an observation related to CHX use, although with a rare frequency. The condition usually subsides spontaneously within a few days after discontinuing use, as was also observed in the present adverse event. The clinical features are suggestive of mechanical obstruction of the parotid duct. It has been suggested that over-vigorous mouthrinsing may predispose to this effect (56).

The results of an *'in-vitro'* study suggested that the activity of CPC would be affected when used as an adjunct to dentifrice (21). It supported the concept of avoiding the use of antiseptic mouthrinses until some time after brushing (57). An *'in-vivo'* study (58) combining the use of CPC with a dentifrice slurry substantiated the observed *'in-vitro'* effect. However, as the authors explained, the use of a slurry is not truly representative of the 'real life situation' of toothbrushing with dentifrice. Dentifrice ingredients such as sodium lauryl sulphate (SLS) have shown to inhibit the activity of CHX (57, 59) using a similar study design as for the CPC findings (58). Van Strydonck *et al.* (60–62) tested the effect of the SLS detergent on CHX in an 'everyday oral hygiene situation', i.e. toothbrushing with an SLS-containing dentifrice. As in daily life, the panellists expectorated the remnants of the dentifrice and rinsed with water immediately after brushing with the dentifrice. This purportedly cleared the oral cavity of the residual SLS dentifrice. This latter supposition was confirmed by the results of the Van Strydonck *et al.* (62), which showed that compared with the CHX only group, the dentifrice group showed no reduction in plaque inhibition. Most likely, the same holds true for CPC where a thorough rinsing following brushing will minimize the counter activity of the SLS detergent in dentifrice.

In the United States, CPC is available in two concentrations: 0.05% and 0.07%. The formulation of the active agent in a mouthrinse is extremely important to maintain its bioavailability, biofilm penetrability and substantivity as well as clinical activity (16, 20, 23). Because the positively charged hydrophilic region of CPC is critical to antimicrobial activity, mouthrinse formulations should not contain ingredients that diminish or

compete with the activity of this cationic group. If the formulation is improperly prepared, inactivation of CPC is likely to occur as a result of chemical reactions, complexing, micelle formation or other sources of deactivation. It is recommended that the bioavailability of CPC in each formulation should be determined to reduce such a possibility (63). Stookey *et al.* (25) conducted a 6-month trial among 298 participants to investigate the long-term anti-plaque and antigingivitis benefits of two high bio-available CPC rinse formulations. Relative to placebo, both CPC rinses (0.07% and 0.10%) provided significant reductions in gingivitis (20–23%), gingival bleeding (27–30%) and plaque (17–19%). Numerous short-term trials provide additional evidence of the benefits of CPC rinses that are formulated to meet the FDA criteria for therapeutic rinses. Additional plaque and gingivitis trials have shown that 0.07% high bio-available CPC rinse provides anti-plaque and anti-gingivitis benefits comparable with a positive control mouthrinse containing essential oils (26, 27). The anti-plaque effect of the high bio-available, alcohol-free CPC rinse in this study demonstrates that it is not different from hexetidine mouthrinse. It is suited for a broad range of patients, particularly those sensitive to products containing alcohol.

Conclusion

A 0.07% CPC mouthrinse proved to be effective in inhibiting 'de novo' plaque formation to an extent similar to that of a 0.1% hexetidine product. Compared with hexetidine, the taste of the CPC was appreciated better and less oral sensations following rinsing were observed.

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