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Efficacy of a 0.15% benzydamine hydrochloride and 0.05% cetylpyridinium chloride mouth rinse on 4-day de novo plaque formation

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Abstract

Objective: To evaluate the effect of a mouth-rinse formulation combining benzydamine hydrochloride and cetylpyridinium chloride (BNZ+CPC) in preventing de novo plaque formation, in comparison with CPC and placebo mouth rinses.

Patients and Methods: This was a controlled, observer-blind, cross-over study. In this model of plaque re-growth, subjects received a session of oral prophylaxis and were directed to withdraw oral hygiene measures for the next 4 days, using only the mouth rinse assigned. The outcome parameters were the plaque index (PlI) and gingival index (GI). In addition, microbiological evaluation of the subgingival microflora, by means of culture, was performed, as well as patient-based variables. Data analysis was carried out using ANOVA for Latin-square design.

Results: The analysis of variance showed a significant statistical difference between the BNZ+CPC association and placebo ($p < 0.0001$). No differences between CPC and placebo were detected considering multiple comparisons between treatments. The 90% confidence interval of the differences between BNZ+CPC and CPC showed no equivalence between treatments, being the PlI lower in the BNZ+CPC group. No significant difference between groups in GI was observed. Mean anaerobic colony-forming units (CFU) demonstrated a significant increase between visits in all groups ($p < 0.001$) and differences among groups were not significant. Subjects treated with BNZ+CPC frequently reported ''tingling mouth'' and ''numbness mouth''.

Conclusion: Within the limitations of the study model, the BNZ+CPC combination showed a statistically significant plaque-inhibitory capacity, as compared with the placebo mouth rinse, and an additive effect as compared with CPC. No relevant clinical or microbiological adverse effects were detected.

Key words: antiseptics; benzydamine; cetylpyridinium chloride; dental plaque; de novo plaque formation; microbiology

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Dental plaque is a bacterial biofilm adhering to the tooth surfaces. It is mainly composed of complex bacterial populations organized in a carbohydrate matrix also containing a small number of epithelial cells, leukocytes, macrophages and inorganic components such as calcium and phosphorus. This biofilm structure confers these bacterial populations a high resistance to most chemical anti-bacterial compounds and makes the use of mechanical oral hygiene procedures such as tooth brushing, dental flossing and inter-dental brushing the most effective method for plaque removal. However, the use of anti-microbial compounds in mouth-rinse formulations can play an important role in maintaining oral health by preventing plaque formation, and thus, preventing gingivitis and caries.

Both benzydamine hydrochloride (BNZ) and cetylpyridinium chloride (CPC) are well-known drugs that are

widely diffused worldwide and have extensive clinical and safety data available.

BNZ has demonstrated an anti-inflammatory effect by inhibiting the production and activity of mediators involved in the inflammatory process, as well as by stabilizing the biological membranes of platelets and other pro-inflammatory cells. Topical benzydamine also exhibits local anaesthetic activity (Turnbull 1995), anti-microbial and anti-fungal activity in vitro (Fanaki & El Nakeeb 1992, Pina-Vaz et al. 2000) as well as a plaque-inhibitory effect (ACRAF Italian patient no. 01244714, 1991). In a mouth-rinse formulation, its use has been indicated in the treatment of recurrent aphthous stomatitis (Matthews et al. 1987, Edres et al. 1997), burning mouth syndrome (Sardella et al. 1999), pharyngodynia or sore throat (Passali et al. 2001), and radiation-induced oral mucositis (Epstein & Stevenson-Moore 1986, Lever et al. 1987, Schubert & Newton 1987, Samaranayake et al. 1988, Epstein et al. 1989, 2001). Formulated as a spray, it has been indicated for the treatment of pain after tonsillectomy (Young 1987, Valijan 1989).

CPC is a quaternary ammonium compound with properties and uses typical of cationic surfactants. It exhibits anti-microbial activity against many oral bacteria. Because of its surface-active properties, it has a prolonged activity in the oral cavity as it is bound to the glycoproteins covering the teeth and oral mucosa. As required by the guidelines of the American Dental Association (Council on Dental Therapeutics 1986), it has an insignificant effect on the composition of the normal oral microbiota. Studies evaluating 0.05% CPC formulations have shown variable results for plaque-inhibitory and anti-plaque capacity. Classic studies with home use for 14 days revealed reductions of plaque but not of gingivitis (Ciancio et al. 1978); however, a 6-month home-use study using a new formulation reported both plaque-inhibitory and anti-plaque capacity (Allen et al. 1998). This discrepancy in the results can be owing to differences in formulations, and its use either before or after tooth brushing, as its use prior to tooth brushing does not seem to be useful (Moran & Addy 1991) and if used immediately after brushing with a toothpaste, its activity could be inhibited by the toothpaste formulation (Sheen et al. 2003). CPC has also been evaluated in plaque re-growth models, demonstrating limited benefits as compared with chlorhexidine mouth rinses (Jenkins et al. 1994, Harper et al. 1995, Moran et al. 2000). Combinations of CPC with other active agents have been proposed and tested. Combined with essential oils, it has demonstrated improved results (Hunter et al. 1994, Moran et al. 1994); combined with 0.12% chlorhexidine, it has shown better results than other 0.12% chlorhexidine formulations, both in vitro, in saliva studies, and on de novo plaque formation (Quirynen et al. 2001, Herrera et al. 2003); and combined with 0.05% chlorhexidine and zinc lactate, it has shown a significant effect on oral halitosis parameters (Roldán et al. 2003, Winkel et al. 2003), and on short-term plaque-inhibitory and anti-plaque efficacy in patients in supportive periodontal care (Santos et al. 2004).

The primary objective of the present study was to evaluate the effect of a mouth-rinse formulation combining BNZ+CPC in preventing de novo plaque formation, in comparison with CPC alone and placebo mouth rinses. The secondary objectives were to assess the effect of the association BNZ+CPC on the gingival tissues and periodontal microflora, and to compare the tolerability of these mouth-rinse formulations.

Patients and Methods

Study population

Inclusion criteria

Subjects were enrolled in this study if they met all the following criteria:

- male or female subjects within 18–39 years of age;
- periodontally healthy subjects;
- at least 22 permanent teeth suitable for plaque and gingival recordings on buccal and lingual surfaces;
- absence of fixed or removable prostheses, and orthodontic appliances;
- subject wishing to actively participate in the study;
- IRB approved informed consent form signed and dated by the subject;
- non-smokers or former smokers (quitted at least 6 months before the trial).

Exclusion criteria

Subjects were excluded from the study if they met any of the following criteria:

- use of systemic or topic (oral) anti-microbial therapy in the previous 2 months;
- presence of clinically significant disease that might compromise the study;
- hypersensitivity to any of the components of the tested medications;
- participation in another trial involving any investigational drug during the past 60 days;
- pregnancy, lactation.

Study design

The present study was a Phase I, single centre, observer-blind, cross-over, randomized Latin-square-controlled design with at least 10 days of wash-out between two consecutive treatments. Two controls were used, a positive control, containing CPC, and a negative control (placebo). Subjects were randomly assigned to receive one of the six possible sequences of treatments.

Seven visits were scheduled. Subjects were treated for 4 days in each session, with a 10-day wash-out period. The length of the experimental period was 43 days, including Visit 0.

Initial appointment

Before starting the experimental phase, the investigator performed a careful evaluation of the medical conditions and oral status of the subject. When subjects met the inclusion/exclusion criteria, the investigator informed him/her on the objectives and details of the study and requested his/her voluntary participation. After signing the IRB approved informed consent form, the subject entered the study.

The investigator assigned each subject a code number given according to the order of the subject's enrolment and assigned each subject the first free randomization number of the randomization list.

Visit 0 (day −10)

Each subject received an oral professional prophylaxis to remove all plaque, calculus and stain on the teeth. Subjects were then provided with a kit for oral hygiene containing a dental floss, a standard toothbrush and a conventional toothpaste, without fluoride or any other

active ingredients, and were instructed to use it after every meal until the next visit.

Subjects were then instructed to follow their normal diet and not to rinse with any anti-microbial compound.

Visit 1 (day 1)

Ten days after Visit 0, subjects had an oral examination to verify the accomplishment of the inclusion criteria (including the oral health of subjects). After the gingival index (GI) assessment, a microbiological sample was collected. Subjects received a session of oral prophylaxis to remove any plaque, calculus and stain, and were given a supply of the allocated formulation. Then, the subjects were instructed to withdraw from any oral hygiene measures for the following 4 days. During this period, subjects were instructed to follow their normal diet, to avoid the use of chewing gum and to use the allocated products. The assigned mouth-rinse regime was the only oral hygiene procedure allowed during the experimental period.

Visit 2 (day 5)

After the last morning rinse in day 5, a final visit (Visit 2) was carried out. The GI and plaque index (PlI) were subsequently assessed and a microbiological sample was collected.

In order to assess the safety of the tested mouth rinse, subjects were asked whether they had had tingling, taste alteration, dry mouth and burning sensation. Based on these answers the investigator recorded a clinical tolerability rating.

In order to assess compliance, containers with the remaining medication were collected by the investigator after the 4-day use and measured.

After the oral examination, patients had an oral prophylaxis to remove any plaque, calculus and stain on the teeth. Subjects were then asked to resume their oral hygiene regimen as described in Visit 0.

Visits 3 and 4 and Visits 5 and 6 followed the same protocol after the 10-day wash-out period and the randomized allocation of new mouth-rinse formulation described in the cross-over design.

Treatments

Dosage and administration procedures

For each treatment period, subjects rinsed three times per day for 4 days, according to the following daily regimen: 15 ml for 1 min. in the morning (after breakfast); 15 ml for 1 min. in the afternoon (after lunch); 15 ml for 1 min. in the evening (after dinner). Rinsing was performed after meals and no food was allowed immediately after (until 30 min.).

Treatment products

The Sponsor provided the following investigational formulations:

- BNZ+CPC mouth rinse (0.15% BNZ+0.05% CPC);
- CPC mouth rinse (Periogard® Plus, Colgate-Palmolive, Piscataway, NJ, USA, 0.05% CPC);
- placebo mouth rinse (0.0% BNZ+CPC, otherwise identical to BNZ+CPC mouth rinse, including 8.1% of alcohol).

Suitable labels were attached to each package of the study samples. Each subject was supplied a total of three bottles of mouthwash, one for each of the three formulations under investigation. Subjects were randomly assigned to sequences, using one randomization list, generated with an SPSS/pc version 8.0 program.

Because of different aspects (colouring and packaging) of the study mouth rinse, a double-blind design was not feasible. For this reason, the study was performed according to an observer-blind design.

Treatment compliance

Subjects received one bottle of mouth rinse for each formulation at each session. The investigational product accountability was checked at Visits 2, 4 and 6.

Treatment compliance was assessed by measuring the volume of product returned. The subject was considered testable if his/her compliance was $\geqslant 80\%$.

Outcome variables

Efficacy outcome parameters

The primary outcome parameter was the PlI. PlI was assessed by means of the Turesky et al. (1970) modification of the Quigley & Hein (1962) index at Visits 2, 4 and 6. Scoring of plaque was performed after staining with erythrosin solution (Red Cote, Butler, Montvale, NJ, USA). The solution was selectively applied with cotton pellets on six sites of the six Ramfjord teeth (16, 11, 24, 36, 31, 44) (Rams et al. 1993). Then the subject was asked to thoroughly rinse with 20 ml of tap water for 15 s. The scores (PlI for the site) from the six sites of the tooth were added and divided by six to give the PlI for the tooth. Adding the indices for the examined teeth and dividing by the total number of examined teeth, the PlI for the subject was obtained.

The GI (Loe & Silness 1963) was evaluated at Visits 1–6, on six sites of the six Ramfjord teeth, as it may exert an influence on the extent of supragingival plaque accumulation (Quirynen et al. 1991). The scores (GI for the area) from the six sites of the tooth were added and divided by six to give the GI for the tooth. Adding the indices for the examined teeth and dividing by the total number of examined teeth, the GI for the subject was obtained.

Microbiological evaluation

In order to assess the effect of the tested mouth rinses on the oral microflora, a microbiological evaluation of plaque samples was carried out for each treatment period. Pooled samples of four sites were collected at baseline and at the end of each test period.

The selected sites were the mesiobuccal locations of the first molars, or the second molar, if the first molar was not present.

Plaque samples were collected immediately after the GI assessment and before the prophylaxis (Visits 1, 3 and 5), and after GI and PlI assessment, 30 min. after the last rinse and before the prophylaxis (Visits 2, 4 and 6).

Two consecutive paper points (number 30, cellpacked, Denstply-Maillefer, Ballaigues, Switzerland) were inserted in the gingival sulcus, and kept in place for 10 s. They were then transferred to a vial containing 1.5 ml of reduced transport fluid (RTF), and transported to the laboratory within 2 h, where they were dispersed (30 s of vortexing), serially diluted and plated on two different media:

- Blood agar medium (Oxoid no 2; Oxoid Ltd., Basingstoke, UK), with 5% horse blood, and with haemin (5 mg/l) and menadione (1 mg/l);
- Dentaid-1 medium (Alsina et al. 2001).

Blood agar plates were studied after 7 and 14 days of anaerobic incubation

(80% N_2, 10% H_2, 10% CO_2 at 37°C), and Dentaid-1 plates after 3–5 days of incubation at 37°C in air with 5% CO_2.

Total microbial counts were evaluated on blood agar plates. On these plates, *Porphyromonas gingivalis*, *Prevotella intermedia/nigrescens*, *Tannerella forsythensis*, *Peptostreptococcus micros*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Capnocytophaga* sp., *Eikenella corrodens* and *Prevotella melaninogenica* were identified, as well as any other representative colony, primarily based on colony morphology, and the use of different tests to confirm the initial identification. Colonies were counted and the percentage corresponding to the total flora for each pathogen was calculated.

Actinobacillus actinomycetemcomitans was identified, when present, on Dentaid-1 plates, based on colony morphology and positive catalase reaction.

Moreover, microbiological plates were studied to detect overgrowth of opportunistic species, in order to assure the safety of the tested products in regard to undesired microbiological adverse effects.

Side effects

To determine safety, each subject was questioned regarding possible mouthrinse side effects: tingling, taste alteration, dry mouth and burning sensation. For each side effect, subjects were asked to give a score according to the following scale: 0 = absent; 1 = mild; 2 = moderate; 3 = severe. This evaluation was performed at the final visit for each treatment period (Visits 2, 4 and 6).

At the final visit for each treatment period, the Investigator expressed a Clinical Rating of Tolerability as follows: 0 = very good (no adverse drug reaction); 1 = good (slight adverse drug reaction spontaneously resolved); 2 = fair (adverse drug reaction requiring corrective treatment); 3 = poor (adverse drug reaction requiring the discontinuation of the test drug); 4 = very poor (serious adverse drug reaction).

Data analyses

Sample size calculation

The aim of the present trial was to demonstrate the equivalence of the association BNZ+CPC with respect to CPC alone by a non-inferiority trial, and the superiority of the association BNZ+CPC with respect to placebo in the inhibition of de novo plaque formation. In previous studies (Moran et al. 2000), the PlI comparison between CPC and placebo has shown a mean difference of 0.66 with a standard deviation of 0.53. Fixing the threshold to 0.33 (50% of the effect with respect to placebo), a sample size of 24 subjects will provide a power of 90% ($\alpha = 0.05$, one sided) in demonstrating that, if the hypothesis of the equivalence holds, the lower confidence limit of the difference between BNZ+CPC minus CPC alone does not exceed the threshold of -0.33. This sample size provides a power higher than 99% ($\alpha = 0.05$, two sided) in demonstrating the superiority of the association BNZ+CPC with respect to placebo.

Two populations were considered for analyses. The intent-to-treat (ITT) population included all subjects randomized and the protocol population (PP), which excluded patients with major protocol violations.

PlI was the main outcome variable. As only one set of data was available per group (PlI was only recorded at the end of each experimental period), an inter-group comparison was performed. It was evaluated by an analysis of variance following a Latin-square design including period, sequence, subject within sequence and treatment effects as sources of variation. The presence of a sequence effect was tested against subject within sequence error, while the other sources of variation were tested against the residual error. The least-square means statement of PROC GLM was implemented to test the superiority of BNZ+CPC with respect to placebo and to calculate the one-sided 95% confidence interval (CI) of the difference between BNZ+CPC and CPC alone in order to demonstrate the non-inferiority hypothesis. In addition, a comparison between CPC and placebo with Dunnett's test was performed.

GI was compared following the same path, but in addition, final assessments were compared with baseline.

Microbiological variables were analysed in different ways:

- Total anaerobic colony-forming units (CFU) were log transformed to fit a normal distribution, and processed by means of ANOVA to compare all groups, identifying differences by means of the multiple-rank test. Secondly, pairs of groups were compared by paired *t*-test. Both tests were performed after evaluating the normal distribution of the samples. If normality was not present, a Kruskal–Wallis test (instead of ANOVA), or a signed-rank test, was chosen.
- CFU of each pathogen were log transformed, given a convenience value of 9 to ''under the level of detection'' category. Only intra-group comparisons were performed, by paired *t*-test.
- The percentage of flora for each pathogen was calculated. Inter-group comparisons were carried out using a Kruskal–Wallis test, and intra-group assessment by means of a signed-rank test.
- Finally, the frequency of detection of each pathogen was compared (only intra-group) by means of a chi-squared test.

Clinical Tolerability Rating and Subjective tolerability evaluation were analysed by two-tailed Wilcoxon's signed-rank test, and the *p*-values were adjusted for the number of the comparisons performed at 0.05 level of significance.

Results

Twenty-four subjects were enrolled in the trial. One subject underwent a 10-day treatment with amoxicillin (owing to bronchitis). This subject was excluded from the PP population for the efficacy assessment and only included in the ITT analysis. Thus, the PP population included 23 patients, while the ITT population consisted of 24 subjects. Clinical- and patient-based results were studied in the ITT population, while the microbiological results were analysed in the PP population.

Table 1 shows the demographic data of the study. Twenty-four subjects (six males, 18 females) with mean age of 23.4 years (range 21–36 years) were

Table 1. Demographic features of the ITT population ($n = 24$)

Gender		Race	
Female	18	Caucasian	23
Male	6	Hispanic	1
Weight (kg)		Height (cm)	
Mean	59.3	Mean	167.7
SD	12.35	SD	7.16
Range	48–92	Range	160–181
Age (years)			
Mean	23.4		
SD	3.71		
Range	21–36		

ITT, intent-to-treat.

Table 2a. Mean plaque index (and standard deviation) for the ITT population ($n = 24$)

	BNZ+CPC	CPC	Placebo
Mean	1.972	2.238	2.397
SD	0.562	0.483	0.506

ITT, intent-to-treat; BNZ, benzydamine hydrochloride; CPC, cetylpyridinium chloride.

enrolled. All subjects were systemically and periodontally healthy with at least 22 teeth free of prosthetic or orthodontic appliances.

Compliance

The treatment compliance was verified by checking returned materials.

PII

All groups increased plaque levels during the experimental periods. The increase in the BNZ+CPC group was the lowest, followed by the CPC group and the placebo group (Table 2a). The analysis of variance showed a significant statistical difference between the BNZ+CPC association and placebo ($p < 0.0001$), in terms of lower de novo plaque formation observed in the BNZ+CPC treatment group. Moreover, the 90% CI of the difference between BNZ+CPC and CPC showed no equivalence between treatments, being the PlI value detected with BNZ+CPC significantly lower than that observed with CPC alone (Table 2b) (90% CI from -0.41 to -0.12, ITT population). No differences between CPC and placebo were detected in the ITT and PP populations, considering multiple comparisons between treatments.

GI

Mean GI demonstrated a clear and significant increase during the experimental periods in all groups ($p < 0.05$). No differences between groups were detected (Table 3).

Microbiological Variables

Mean anaerobic CFU, log transformed, demonstrated a significant increase between visits in all groups ($p < 0.001$). Differences among groups were not significant, both evaluated by ANOVA or by paired *t*-test (Table 4).

In regard to the presence of periodontal pathogens, *T. forsythensis* and *A. actinomycetemcomitans* were not found in any sample at any visit. *P. gingivalis* was only found in two samples from the same patient (baseline visits of CPC and placebo series). Table 5 shows the results on the most prevalent species. *F. nucleatum* was detected in almost all the samples. The mean log of CFU demonstrated a significant increase in all groups ($p < 0.001$). The mean percentage of the flora also increased in all groups, reaching the level of significance for the CPC and placebo groups ($p < 0.05$). No significant differences were detected among groups.

C. rectus showed minor changes in the frequency of detection. The mean log-transformed CFU increased in all groups, but not significantly. The percentage of flora demonstrated no changes.

Capnocytophaga sp. increased its prevalence in all groups, especially in CPC and placebo groups. The mean log of CFU also increased, reaching the level of significance for CPC and placebo groups ($p < 0.01$). The proportion of flora increased significantly in the placebo group ($p = 0.01$). No inter-group differences were detected.

P. intermedia/nigrescens demonstrated minor variations in prevalence, except the BNZ+CPC group that showed an important reduction. The mean log of CFU clearly increased in all groups, reaching the level of significance in the placebo group ($p = 0.001$). The percentage of flora showed a reduction in the BNZ+CPC group, and minor changes in the other groups. The inter-group comparison showed a tendency towards significance at final visit ($p = 0.05$), corresponding to a higher level of placebo as compared with the other two groups.

E. corrodens demonstrated an increase in prevalence in all groups. The same was true for the mean log of CFU, being statistically significant for groups BNZ+CPC and placebo ($p < 0.05$). The mean percentage of flora clearly increased in the placebo and CPC groups. No inter-group differences were detected.

P. micros showed minor changes in frequency of detection. The mean log of CFU increased, but not reaching the level of significance. Mean proportions decreased in BNZ+CPC group,

Table 2b. Differences in plaque index between groups (mean and 90% confidence interval), for both the ITT and PP populations

	ITT ($n = 24$)		PP ($n = 23$)	
	mean difference	90% CI	mean difference	90% CI
BNZ+CPC *versus* CPC	-0.27	$(-0.41;\ -0.12)$	-0.26	$(-0.40;\ -0.11)$
BNZ+CPC *versus* placebo	-0.42	$(-0.57;\ -0.28)$	-0.45	$(-0.60;\ -0.30)$
CPC *versus* placebo	-0.16	$(-0.31;\ -0.01)$	-0.19	$(-0.34;\ -0.04)$

Negative means a higher value of the group mentioned in the second place.
ITT, intent-to-treat; PP, protocol population; BNZ, benzydamine hydrochloride; CPC, cetylpyridinium chloride; CI, confidence interval.

Table 3. Mean gingival index (and standard deviation) for the ITT population ($n = 24$)

	Baseline		Final		Difference (baseline − final)	
	mean	SD	mean	SD	mean	SD
BNZ+CPC	0.201	0.169	0.520	0.487	-0.319	0.519
CPC	0.168	0.137	0.455	0.403	-0.287	0.422
Placebo	0.210	0.153	0.447	0.374	-0.237	0.415

ITT, intent-to-treat; BNZ, benzydamine hydrochloride; CPC, cetylpyridinium chloride.

Table 4. Mean log of total anaerobic counts, per group and visit (PP population, $n = 23$)

	Baseline		Final		Difference (baseline − final)	
	mean	SD	mean	SD	mean	SD
BNZ+CPC	4.799	0.599	5.986	0.479	-1.187	0.676
CPC	4.796	0.668	6.012	0.333	-1.216	0.611
Placebo	4.718	0.568	6.022	0.359	-1.304	0.683

PP, protocol population; BNZ, benzydamine hydrochloride; CPC, cetylpyridinium chloride.

Table 5. Frequency of detection, mean and standard deviation of CFU, and mean percentage of bacteria in positive sites, for different bacterial species, per group and visit (PP population, $n = 23$)

	BNZ+CPC		CPC		Placebo	
	baseline	final	baseline	final	baseline	final
Fusobacterium nucleatum						
Frequency of detection (%)	100	96	100	100	100	96
Mean-CFU	5136	91,881	6426	123,621	2578	123,043
SD-CFU	9177	181,515	14,978	93,406	3462	202,744
Mean% (+)	4.5	6.2	2.6	12.0	3.3	8.9
Campylobacter rectus						
Frequency of detection (%)	13	22	22	26	17	17
Mean-CFU	23	1033	387	1406	175	752
SD-CFU	84	3010	1388	3235	570	2021
Mean% (+)	0.6	0.5	0.7	0.7	0.4	0.4
Capnocytophaga sp.						
Frequency of detection (%)	13	22	9	43	13	48
Mean-CFU	204	2095	574	7231	69	33,144
SD-CFU	729	5029	2752	17,989	278	136,839
Mean% (+)	0.8	1.1	1.3	0.9	0.9	2.1
Prevotella intermedia						
Frequency of detection (%)	61	43	52	48	70	61
Mean-CFU	3943	24,263	4423	23,507	756	34,865
SD-CFU	14,061	104,235	16,775	74,373	2145	103,859
Mean% (+)	6.0	1.8	2.4	2.2	1.5	2.5
Eikenella corrodens						
Frequency of detection (%)	9	26	13	17	4	22
Mean-CFU	29	1960	79	1463	29	2382
SD-CFU	138	4580	258	5575	138	7071
Mean% (+)	0.7	0.4	0.3	0.8	0.2	0.6
Peptostreptococcus micros						
Frequency of detection (%)	27	23	26	22	17	22
Mean-CFU	199	2328	115	9768	127	1857
SD-CFU	580	8491	441	43,973	370	5744
Mean% (+)	1.8	0.7	0.5	1.2	1.4	1.4

PP, protocol population; BNZ, benzydamine hydrochloride; CPC, cetylpyridinium chloride; CFU, colony-forming units.

Table 6. Adverse effects as defined by the patients after use (ITT, $n = 24$)

	Tingling		Variation in taste		Dry mouth		Burning	
	n	%	*n*	%	*n*	%	*n*	%
BNZ+CPC								
Absent	6	25.0	22	91.7	23	95.8	20	83.3
Mild	5	20.8	0	0.0	0	0.0	0	0.0
Moderate	10	41.7	2	8.3	1	4.2	3	12.5
Severe	3	12.5	0	0.0	0	0.0	1	4.2
CPC								
Absent	19	79.2	21	87.5	24	100.0	20	83.3
Mild	5	20.8	1	4.2	0	0.0	1	4.2
Moderate	0	0.0	2	8.3	0	0.0	3	12.5
Severe	0	0.0	0	0.0	0	0.0	0	0.0
Placebo								
Absent	19	79.2	24	100.0	24	100.0	23	95.8
Mild	3	12.5	0	0.0	0	0.0	0	0.0
Moderate	2	8.3	0	0.0	0	0.0	1	4.2
Severe	0	0.0	0	0.0	0	0.0	0	0.0

ITT, intent-to-treat; BNZ, benzydamine hydrochloride; CPC, cetylpyridinium chloride.

increased in CPC group and kept identical values in placebo group, but none of these changes, nor the inter-group assessments, were statistically significant.

Adverse Events

Subjects reported their mouth feelings after using every product, and qualified them using a verbal scale (Table 6). A ''tingling'' feeling was the most frequent complain in BNZ+CPC patients, affecting 75% of them, compared with 21% in the other groups. Moreover, 54% reported tingling as moderate or severe after using the BNZ+CPC product. Conversely, variation in taste, dry-mouth or a burning feeling was rarely referred.

Clinical Tolerability

The clinical tolerability rating expressed by the Investigator was always good or very good (Table 7). However, the Wilcoxon signed-rank test showed a statistically significant difference between BNZ+CPC *versus* CPC and placebo.

Discussion

PII

The results of the present study demonstrate that, within the limitations of the selected study model, the tested mouthrinse formulation containing BNZ+CPC was significantly more efficient

Table 7. Valuation of the tested products by the researchers

	BNZ+CPC		CPC		Placebo	
	n	%	*n*	%	*n*	%
Very good	2	8.3	12	50.0	18	75.0
Good	22	91.7	12	50.0	6	25.0
Fair	0	0.0	0	0.0	0	0.0
Poor	0	0.0	0	0.0	0	0.0
Very poor	0	0.0	0	0.0	0	0.0

BNZ, benzydamine hydrochloride; CPC, cetylpyridinium chloride.

than placebo in the inhibition of plaque formation. Moreover, when compared with a commercialized formulation containing CPC as the active ingredient, BNZ+CPC demonstrated significantly better plaque-inhibiting efficacy. This leads to the conclusion that the combination of BNZ with a standard 0.05% CPC formulation increases the plaque-inhibitory capacity of BNZ. A possible explanation of this result is that benzydamine prevents bacteria from adhering to the teeth surface, as already reported for other cations (Skjorland et al. 1978).

The mean PlI observed in the placebo group was lower than that reported in the literature in similar experimental conditions (Moran et al. 2000). This is probably owing to the fact that in this study the placebo formulation contains all the excipients (the vehicle) of the BNZ+CPC formulation, including 8.1% alcohol. Although a complete inhibition of the plaque biofilm growth requires higher concentrations of alcohol, a potential contribution of the 8.1% alcohol in the reduced plaque re-growth cannot be excluded.

The results for the CPC formulation were worse those that reported in similar studies (Moran et al. 2000), as no significant differences in plaque-inhibitory effect were detected when compared with placebo using ANOVA. As mentioned earlier, these results could be explained by the possible plaque-inhibitory effect of alcohol included in the placebo formulation. However, comparison between CPC and placebo showed a significantly higher effect in the CPC group.

GI

No differences were detected in the level of gingival inflammation. GI was not evaluated as an outcome measure. Its utility in this trial was for controlling as a possible confounding factor influencing plaque accumulation (Quirynen et al. 1991). As no differences were detected among the groups, and no influence was observed when it was included in the ANOVA test as a covariable, it shows no confounding effect in the plaque results.

Microbiological results

Subgingival plaque was analysed for two reasons: firstly, to assess possible subgingival anti-microbial effects of the tested products, and secondly, to evaluate any possible microbiological adverse effects, such as the growth of opportunistic microorganisms.

In regard to the first aim, the total subgingival anaerobic flora clearly increased as supragingival plaque accumulated during the experimental periods, and no differences were detected among the groups. However, when specific bacterial pathogens associated with gingivitis were analysed, some findings were detected for *P. intermedia/nigrescens*, *F. nucleatum* and *Capnocytophaga* sp. suggesting an effect of the BNZ+CPC formulation, as these bacteria showed a lower increase in the mean CFU, a lower increase in their proportion of the total flora, and in their frequency of detection, although no statistically significant differences between groups were found. The explanation for these positive results can be related to the lower amount of supragingival plaque, or to a true subgingival effect. Other oral hygiene products have demonstrated a positive influence in the subgingival microflora composition, both formulated as dentifrices (triclosan-Gantrez®, Colgate-Palmolive) (Rosling et al. 1997), or as mouthrinse formulations (chlorhexidine–CPC) (Roldán et al. 2003, Santos et al. 2004). However, it remains unclear whether this effect is because of a direct effect on the product on the subgingival microflora, or indirectly through its influence on the supragingival plaque.

In regard to the possible adverse microbiological effects, no overgrowth of opportunistic microorganisms was detected in any of the tested groups.

Adverse effects

Adverse effects were assessed in two ways: firstly, by questioning the patient after each experimental period, and secondly, by a subjective evaluation carried out by the investigator.

No differences among groups were observed when patients were asked about changes in taste, burning feeling or dry mouth. However, a very obvious difference was seen when patients were asked about a tingling feeling, related with the use of the BNZ+CPC product. This effect is typically related to the known local anaesthetic activity of benzydamine, which is often confused with an adverse effect, especially by healthy volunteers, while it is a valid therapeutic tool in sore throat and inflammatory conditions of the mouth. It remains unclear whether subjects once informed about this effect would better tolerate this feeling. Previous studies have found the same problem when BNZ alone was used in patients with radiation-induced mucositis (Lever et al. 1987, Samaranayake et al. 1988), or in recurrent aphthous stomatitis (Matthews et al. 1987), even leading to recommend not to use BNZ in patients with severe stomatitis (Lever et al. 1987).

The subjective evaluation carried out by the investigator at the end of each experimental period demonstrated that all products were categorized as good or very good in relation with subject tolerance. However, the problems with the tingling feeling caused by the anaesthetic activity of BNZ lead the researcher to qualify the BNZ+CPC product lower.

Summary

Within the limitations of the selected study model, it can be concluded that the formulation combining BNZ and CPC demonstrated a statistically significant plaque-inhibitory capacity, as compared with the placebo mouth rinse. It also demonstrated an additive plaque-inhibitory activity as compared with a commercial formulation with CPC. No relevant clinical or microbiological adverse effects were detected, although most patients complained of a tingling feeling when using the BNZ+CPC formulation, related with its topical anaesthetic activity.

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