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ORIGINAL ARTICLE

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Rioboo M, García V, Serrano J, O'Connor A, Herrera D, Sanz M. Clinical and microbiological efficacy of an antimicrobial mouth rinse containing 0.05% cetylpyridinium chloride in patients with gingivitis.

Clinical and microbiological efficacy of an antimicrobial mouth rinse containing 0.05% cetylpyridinium chloride in patients with gingivitis

Abstract: *Objectives:* the aim of this study was to evaluate the effects of the use of a mouth rinse and dentifrice with cetylpyridinium chloride (CPC) in patients with gingivitis. *Methods:* the study was designed as a 1-month, double-blind, parallel, randomized clinical trial comparing a negative control regimen (minus active ingredients dentifrice and mouth rinse) with the test products (dentifrice and mouth rinse with 0.05% CPC) in terms of plaque and gingival indexes (PI, GI), patient-based and microbiological outcome variables. The comparisons in relation to the main outcome variables (PI and GI) were made by means of the *t*-test, either unpaired or paired for the intergroup and intragroup comparisons, respectively. *Results:* no differences were detected at baseline. Both groups showed statistically significant decreases in GI (0.17–0.19), without intergroup differences. The PI demonstrated a significant decrease of −0.12 in the test group and minor changes in the negative control group (increase of +0.01). Differences between groups showed a tendency towards statistical significance. A limited impact was observed for microbiological variables in both groups. *Conclusions:* the results of this study show limited benefits of the evaluated formulations as adjuncts to unsupervised oral hygiene in reducing plaque accumulation, and no effect on gingivitis.

Key words: cetylpyridinium chloride; gingivitis; microbiology; mouth rinse; plaque

Introduction

The rationale of underpinning the use of antimicrobial mouth rinses in dentistry is based on the clear demonstration of the relationship between plaque accumulation and the development of gingivitis (1). Gingivitis is a highly prevalent periodontal condition affecting 82% of the children and adolescent populations and nearly half of the adult population (2), and its importance lies in the possibility of evolving to periodontitis (3, 4) and this relationship is currently even more emphasized in the light of the recently demonstrated associations between both gingivitis and periodontitis, with systemic diseases (5). Although the basis of gingivitis prevention lies in oral hygiene measures, the majority of people fail to maintain adequate levels of plaque control (6), which provides a clear rationale for supplementing mechanical plaque control methods with effective antimicrobial mouth rinses with demonstrated plaque inhibitory and antiplaque efficacy.

Numerous clinical trials have demonstrated the effectiveness of different antimicrobial mouth rinses in reducing plaque and gingivitis. Although the capacity for plaque inhibition has been demonstrated for several chemical compounds after mechanical plaque control cessation in short-term study designs (16–94 h) (7), the real efficacy must be evaluated in home-use studies in which the chemical agent is used as an adjunct to mechanical plaque control and compared with a placebo rinse.

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound that shares properties with other cationic surfactants in its adsorption to oral surfaces, although with limited substantivity (8). It is capable of killing gram-positive pathogens and yeast through its interaction with the bacterial membrane function, leakage of cytoplasm material and the ultimate collapse of the intracellular equilibrium (9). Cetylpyridinium chloride is in the category of over-the-counter products and has received a Category I (safe and effective) label by the Food and Drug Administration (FDA) advisory panel in 2004. Cetylpyridinium chloride compounds have demonstrated effectiveness and safety as a plaque inhibitory agent in a range of concentrations between 0.045 and 0.1%, although these agents have not been widely classified as efficacious antiplaque agents (10). This is probably due to the variable results reported depending on the formulation used (11), which may provide different chemical bioavailability of CPC. Therefore, the evaluation of the efficacy of any new CPC-based formulations should be based on properly designed clinical studies.

A newly formulated mouth rinse containing CPC has recently been tested in a plaque re-growth model and has demonstrated efficacy as a plaque inhibitory agent (12). It is therefore the purpose of this investigation to assess whether this efficacy may also be demonstrated in a randomized clinical trial when used as an adjunct to mechanical plaque control in a home study, thus assessing its potential benefits and drawbacks.

The hypothesis of the present investigation was to study whether the use of a CPC-containing mouth rinse and dentifrice provides additional benefits in terms of plaque and gingivitis control, when compared with the use of non-CPC-containing mouth rinse and dentifrice. As secondary objective, we also assessed the effect on the microbiology of the subgingival microenvironment.

Material and methods

Patients attending the Faculty of Odontology at the University Complutense of Madrid were consecutively screened for eligibility for this study, from January 2008 to January 2009. To be considered for inclusion, the patient should have at least 1.75 in the Modified Gingival Index (GI) (13) and at least 1.5 in the Modified Plaque Index (14), as minimum mean values in the Ramfjord teeth. Subjects were excluded if presenting with any relevant systemic disease, presenting an untreated oral condition or if they had been taking antibiotics or anti-inflammatory drugs 1 month before the screening. Once all eligible subjects were informed about the objectives and the protocol of the study, they volunteered to participate in the trial by signing an informed consent, previously approved by the official ethics committee, and by adhering to the study protocol.

The study was designed as a 1-month, single-centre, double-blind, parallel, randomized clinical trial. Neither the participants nor the investigators were aware of the composition of the products, identified by codes kept by the study promoter and only opened when the study was finished. Randomization was balanced in terms of tobacco consumption, using two different randomization blocs, one for non-smokers and another for smokers of more or equal to 10 cigarettes per day. Each subject would receive either the test or negative control products. The allocation of product usage was assigned following a randomization order through a computer-generated sequence, associated with the number the subject received when entering the study. Investigators were unaware of the product allocation.

Two formulations were compared:

- Test, consisting of a mouth rinse (VITIS Encías mouthrinse; Dentaid, Cerdanyola, Spain) formulated with 0.05% CPC as the main active ingredient and also containing zinc lactate, permethol and provitamine B5 and a toothpaste (VITIS Encías tooth-paste; Dentaid) also formulated with 0.25% permethol, 1.0% provitamine B5, 0.05% CPC, 0.25%, zinc lactate and 0.33% sodium fluoride.
- Negative control, consisting of a mouth rinse and a dentifrice similar to the test products but without CPC, zinc lactate, permethol and provitamine B5.

The main outcome variables were gingival inflammation assessed by the GI, using the Lobene *et al.*'s (15) modification of the Löe–Silness index, in six sites per tooth (15) and presence of plaque assessed by the plaque index (PlI) using the Turesky *et al.*'s (14) modification of the Quigley and Hein index, after disclosing dental plaque with erythrosine also in six sites per tooth (Plac Control; Dentaid).

As clinical secondary variables, probing pocket depth (PPD), recession and clinical attachment level (CAL) were measured using the Florida® probe (Florida Probe Corporation, Gainesville, FL, USA). All clinical variables were measured at all teeth present, except third molars and teeth with class V fillings, ill-fitted subgingival restorations and/or fixed prosthesis. Moreover, subgingival microbiological samples were taken at the beginning and at the end of the experimental period to assess the changes in the subgingival microflora.

Tooth staining was evaluated both in extension and in intensity using a modification (16) of the staining index described by Lobene *et al.* (17) through the assessment of standardized photographs of the buccal and lingual anterior dentition (lower jaw). See Table 1.

The subject's compliance and occurrence of adverse effects were evaluated at the end of each study interval through a questionnaire (see Fig. 1) and by the measurement of the product remaining in the returned bottles.

In a screening visit, subjects were evaluated (including gingivitis and plaque examinations) in order to assess suitability for inclusion and exclusion. Patients were informed on the

Table 1. **Modification (16) of the staining index described by Lobene *et al.* (17)**

Code	Area stained	Intensity description
0	No staining	No staining
1	=1/3 of the area	Light
2	>1/3 = 2/3 of the area	Moderate
3	>2/3 of the area	Severe

Mouthrinse flavour:
Pleasant ☐ Neutral ☐ Unpleasant ☐

During the use of the mouthrinse, have you felt taste alterations, metallic flavour,…?
Nothing ☐ A few ☐ A lot ☐

Have you felt burning perception after rinsing out?
Nothing ☐ A few ☐ A lot ☐

Have you felt any mucosa alterations (ulcers, descamation…)?
Nothing ☐ A few ☐ A lot ☐

Have you observed any tongue depapilation?
Nothing ☐ A few ☐ A lot ☐

Have you observed any tongue staining?
Nothing ☐ A few ☐ A lot ☐

Have you observed any teeth staining?
Nothing ☐ A few ☐ A lot ☐

Have you observed any other effects?

_____

Have you ever forgotten, during this four weeks, using the mouthrinse?
YES ☐ NO ☐

If yes, how many times?

_____

Fig. 1. Final questionnaire form.

study design and objectives and voluntarily signed an IRB-approved consent. Within 15 days of the screening examination, a baseline visit was rendered, and clinical variables were measured and subgingival microbiological samples were taken. Subjects were then allocated to one of the two experimental groups and instructed to rinse with 15 ml of the assigned product for 30 s twice daily (after breakfast and dinner) after their unsupervised oral hygiene procedures. Subjects were then supplied with two 500-ml bottles of test or negative control product (all mouth rinses were packed in identical bottles) and a test or negative control tube of dentifrice in a plain white tube. In addition, they received a standard toothbrush (VITIS Encías; Dentaid) and either floss (VITIS Seda Dental suave; Dentaid) or interdental toothbrushes (Interprox; Dentaid). Subjects were asked to return the bottles both unused and with remaining product at the end of the study.

One month later, at the final visit, subgingival samples were again taken, clinical variables were assessed, and the occurrence of adverse effects and compliance were evaluated by a questionnaire (Fig. 1). Subjects received then a prophylaxis and were then allowed to resume their usual oral hygiene practices.

Calibration between examiners was carried out for both the indexes used (GI and PlI) at baseline and at the end of a previous 4-day experimental study (12). Comparisons were made by means of the kappa test and the 95% confidence interval. Regarding the intra-examiner calibration, examiner 1 scored 0.68 (0.58–0.77) for GI and 0.65 (0.56–0.74) for PlI. Examiner 2 scored 0.42 (0.29–0.55) for GI and 0.41 (0.31–0.51) for PlI. Interexaminer calibration results were 0.59 (0.46–0.72) for GI with an agreement of 85% and 0.68 (0.58–0.77) for the PlI with an overall agreement of 80%. Calibration was considered as good for examiner 1 and as moderate for examiner 2. Inter-examiner agreement was good for the main outcome variables of the study.

Microbiological samples were taken using two sterile paper points (Maillefer, Ballaigues, Switzerland) that were inserted consecutively in each selected site (four sites showing clear signs of gingivitis, scores 2 or 3, from two anterior and two posterior teeth) (18). Before the insertion of the paper points, supragingival plaque was removed, the sites were isolated with cotton rolls to avoid saliva contamination, and the area was dried with the syringe from the dental chair. Paper points were kept in place for 10 s and then pooled in a screw top vial containing 1.5 ml of reduced transport fluid (19). Samples were transferred to the laboratory within 2 h where they were homogenized by vortex vibration for 30 s (20) and sequentially diluted in PBS (phosphate-buffered solution). The samples were then cultivated on agar-blood medium (enriched with haemine and menadione) incubated for 15 days in jars with an anaerobic atmosphere and on selective medium Dentaid-1 incubated for 3–5 days in 5% carbon dioxide (21). Bacterial species identification was carried out by the assessment of the colony morphology and confirmed by the application of biochemical standard tests. In addition to the conventional evaluation of the plates, the possible overgrowth of opportunistic species, both in blood agar and in selective plates, was investigated. The main microbiological outcome variables included total anaerobic counts and the presence, counts and proportions of different bacterial species, including opportunistic species in order to detect possible undesired microbiological adverse effects.

Statistical analysis

The sample size was calculated based on the plaque re-growth study previously conducted with the same tested product (12) in which the standard deviation for changes in PlI was 0.72. In order to attain a statistically significant difference (difference

0.75) with a 95% power and taking into account the possible dropouts, it would be necessary to have 30 patients per group.

The patient was the unit of analysis. For the clinical variables, means were calculated first by patient and then by group, at each visit. Comparisons between groups were evaluated by the unpaired Student's *t*-test (after assessing that they fitted a normal distribution). Intragroup comparisons between baseline and 1 month were assessed by the paired *t*-test.

For tooth staining, area and intensity were evaluated separately. For each variable, staining was calculated as the sum of the scores of the six assessed teeth, separately for the buccal and lingual sites. Means of the sums were calculated per visit and group and then compared in a similar way as that described for the clinical variables.

Mean of log of total bacterial counts was calculated and compared as previously described. The same was true for the mean counts of pathogens. The frequency of detection in percentage was compared by means of the chi-square test, and the mean proportions of flora in positive site of different periodontal pathogens were compared by means of the Wilcoxon test.

Results

Ninety consecutive patients were screened and 63 fulfilled the inclusion criteria and accepted to participate (see Fig. 2). In the test group, 30 patients were randomized, but three did not attend the baseline visit and were excluded from the study. This group therefore consisted of 27 patients. In the negative control group, 33 patients were included and attended the baseline visit. Two patients in the control group (two women, one smoker) did not attend the 1-month visit. The intent-to-treat population included 27 test and 33 control subjects, and the per-protocol population, 27 and 31, respectively.

Table 2 shows the participant subject demographics. No significant differences in gender, age or tobacco habits were detected between groups.

Table 3a depicts baseline and 1-month levels of GI, while Table 4 shows changes between visits. Baseline GI values were similar in both groups, ranging between 1.62 and 1.69. The intragroup analysis showed statistically significant decreases in GI ($P < 0.005$), similar in both groups (0.17–0.19 for all sites). Similar results were shown when the analysis was

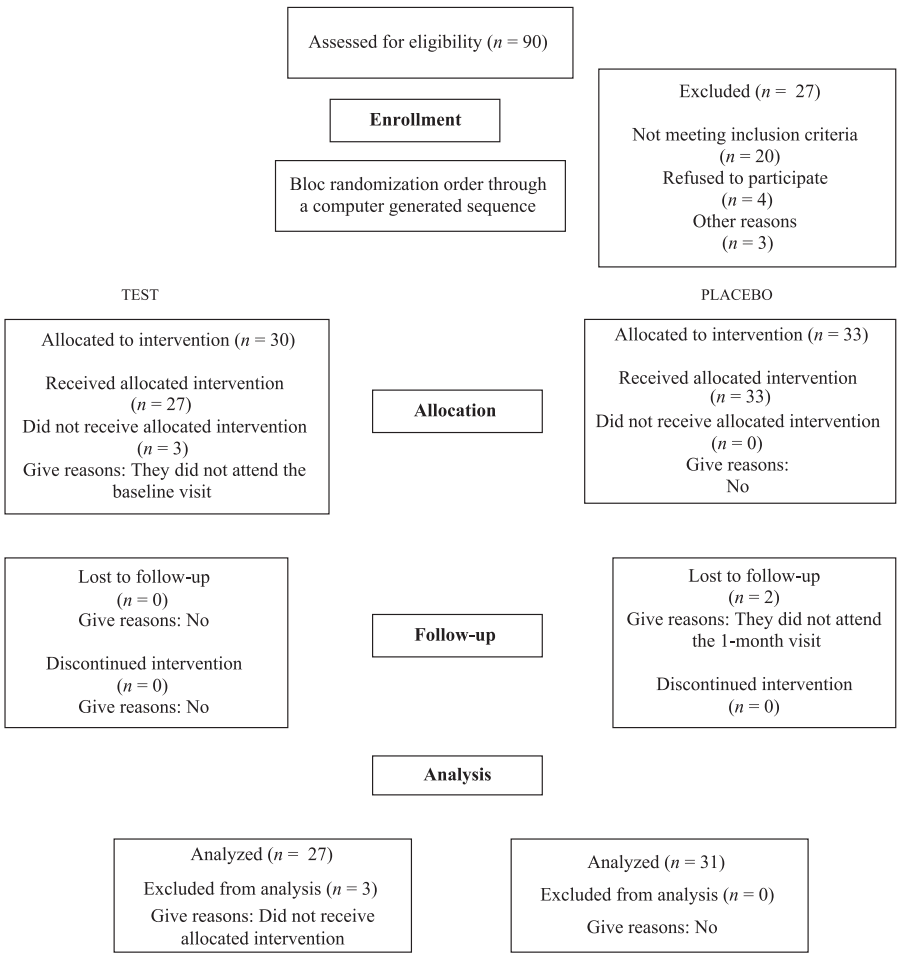

Fig. 2. Flow chart diagram showing screening, enrollment and follow-up of patients during the study.

Table 2. **Demographic characteristics in both study groups**

	Negative control group	Test group	Intergroup
No.	33	27	Chi square
No. of males	16	14	$P = 0.795$
No. of females	17	13	
Mean age	31.9	32.4	*t*-test
Standard deviation	10.0	11.5	$P = 0.858$
Minimum age	60	61	
Maximum age	21	18	
No. of smokers ≥10 cig per day	9	8	Chi square $P = 0.840$
No. of non-smokers	24	19	

stratified for buccal, lingual, proximal or buccal and lingual sites. The comparison between test and negative control groups at 1 month did not demonstrate significant differences, with similar gingivitis levels in both groups (1.43–1.50). No significant differences between groups were also detected in the changes between baseline and 1 month.

Table 3b depicts baseline and 1-month levels of PlI, while Table 4 shows changes between visits. Baseline PlI values were similar in both groups, ranging between 1.62 and 1.69. After 1 month, the PlI for all sites demonstrated a significant ($P = 0.03$) decrease of −0.12 in the test group, while the control group remained almost unchanged (increase of +0.01, $P = 0.90$). Differences between groups showed a tendency towards statistical significance ($P = 0.08$). When stratified by site location, at lingual sites, a significant reduction was observed in the test group (−0.13, $P = 0.01$), versus a minor increase in the control group (+0.03, $P = 0.59$). At these sites, differences between groups were statistically significant ($P = 0.03$). At non-proximal sites, differences between groups were close to statistical significance ($P = 0.07$) with PlI values being significantly lower in the test group at 1 month (−0.21, $P = 0.02$).

Baseline PPD values were similar in both groups, ranging between 2.33 and 2.38, with the majority of sites being between 1 and 3 mm in both groups. At 1 month, there were not statistically significant intergroup differences. Mean changes in PPD values showed minimal variations in the placebo group, while the test group demonstrated a significant reduction ($P = 0.004$). In this group, there were a significant increase in the percentage of PPDs in the range of 1–3 mm ($P = 0.001$) and a decrease of sites within the range of 4–6 mm ($P = 0.001$), as shown in Figs 3 and 4.

Baseline CAL values were similar in both groups, ranging between 2.37 and 2.45, with the majority of sites being between

Table 3. **(a) Gingival index and (b) plaque index at baseline and after 1 month, expressed as mean, standard error (SE) and 95% confidence intervals (95% CI), at all sites or only at buccal, lingual, proximal or non-proximal sites. Intergroup statistical comparison made by means of unpaired *t*-test**

(a)

		Baseline					1 month				
Gingival index		Mean	SE	95% CI		*P* value	Mean	SE	95% CI		*P* value
All sites	Control	1.62	0.06	1.54	1.71	0.462	1.44	0.07	1.34	1.53	0.545
	Test	1.69	0.07	1.59	1.78		1.50	0.08	1.39	1.60	
Buccal sites	Control	1.59	0.06	1.50	1.68	0.497	1.38	0.07	1.28	1.49	0.683
	Test	1.66	0.07	1.56	1.76		1.43	0.08	1.31	1.54	
Lingual sites	Control	1.65	0.06	1.56	1.74	0.492	1.49	0.07	1.38	1.59	0.458
	Test	1.72	0.07	1.62	1.82		1.57	0.08	1.46	1.68	
Proximal sites	Control	1.73	0.06	1.65	1.81	0.542	1.56	0.07	1.46	1.66	0.675
	Test	1.78	0.07	1.69	1.88		1.60	0.07	1.50	1.71	
Non-proximal sites	Control	1.41	0.07	1.31	1.51	0.400	1.19	0.08	1.08	1.30	0.368
	Test	1.50	0.08	1.39	1.61		1.29	0.08	1.17	1.41	

(b)

		Baseline					1 month				
Plaque index		Mean	SE	95% CI		*P* value	Mean	SE	95% CI		*P* value
All sites	Control	3.82	0.08	3.71	3.92	0.566	3.81	0.08	3.69	3.92	0.566
	Test	3.86	0.08	3.74	3.98		3.74	0.08	3.62	3.86	
Buccal sites	Control	3.73	0.08	3.62	3.84	0.875	3.69	0.08	3.58	3.81	0.875
	Test	3.78	0.09	3.66	3.90		3.67	0.09	3.55	3.80	
Lingual sites	Control	3.90	0.09	3.78	4.02	0.382	3.92	0.09	3.79	4.04	0.382
	Test	3.93	0.09	3.80	4.07		3.80	0.09	3.67	3.94	
Proximal sites	Control	4.61	0.09	4.49	4.74	0.776	4.60	0.09	4.47	4.73	0.776
	Test	4.64	0.10	4.50	4.78		4.56	0.10	4.43	4.70	
Non-proximal sites	Control	2.22	0.10	2.08	2.36	0.423	2.19	0.10	2.06	2.33	0.423
	Test	2.29	0.11	2.14	2.44		2.08	0.10	1.93	2.23	

Table 4. **Changes between baseline and 1-month visits in gingival and plaque indexes, expressed as mean, standard error (SE) and 95% confidence intervals (95% CI), at all sites or only at buccal, lingual, proximal or non-proximal sites. Statistical comparison made by means of unpaired *t*-test for intergroup comparisons (inter) and by paired *t*-test for intragroup comparisons (intra)**

		Gingival index						Plaque index					
		Mean	SE	95% CI		Intra	Inter	Mean	SE	95% CI		Intra	Inter
All sites	Control	−0.17	0.04	−0.23	−0.11	0.001	0.690	0.01	0.05	−0.06	0.07	0.904	0.083
	Test	−0.19	0.04	−0.25	−0.13	0.000		−0.12	0.05	−0.19	−0.04	0.030	
Buccal sites	Control	−0.19	0.05	−0.26	−0.12	0.001	0.563	−0.02	0.06	−0.10	0.06	0.737	0.309
	Test	−0.23	0.05	−0.31	−0.16	0.000		−0.10	0.06	−0.19	−0.02	0.123	
Lingual sites	Control	−0.14	0.04	−0.20	−0.08	0.005	0.936	0.03	0.05	−0.04	0.10	0.594	0.030
	Test	−0.15	0.05	−0.21	−0.08	0.001		−0.13	0.05	−0.20	−0.06	0.010	
Proximal sites	Control	−0.15	0.04	−0.21	−0.09	0.002	0.650	0.01	0.06	−0.07	0.10	0.823	0.301
	Test	−0.18	0.05	−0.25	−0.12	0.000		−0.08	0.06	−0.16	0.01	0.214	
Non-proximal	Control	−0.19	0.05	−0.26	−0.13	0.001	0.810	0.01	0.06	−0.07	0.10	0.578	0.075
	Test	−0.21	0.05	−0.28	−0.14	0.000		−0.08	0.06	−0.16	0.01	0.025	

1 and 3 mm in both groups. At 1 month, there was a statistically significant mean CAL gain in the test group (P = 0.004) as compared with only minor changes in the placebo group (P = 0.703). When comparing both groups, there was a statistically significant difference in mean CAL values at 1 month (P = 0.045). Also changes in CAL demonstrated significant differences (P = 0.034) between test and placebo groups.

With regard to the microbiological results, from the 30 test subjects, three baseline samples could not be taken and one could not be processed, resulting in 26 available baseline samples. At 1 month, four samples were not taken and one could not be processed, thus resulting in 25 1-month samples and 25 patients with both baseline to 1-month samples.

From the 33 negative control subjects, two baseline samples were not taken and three could not be processed; thus, 28 baseline samples were available in the placebo group. After 1 month, samples were not taken from two patients and five samples could not be adequately processed; therefore, 26 1-month samples and 25 patients with both baseline to 1-month samples were available.

Figure 5 depicts the changes in total bacterial counts between baseline and 1 month. No differences were observed at baseline or at the final visit between both groups. Both study groups showed a small increase in counts from baseline to 1 month, which was higher in the placebo group, but differences were not statistically significant.

Table 5 and Fig. 6 show the changes in the frequency of detection, counts and proportions of selected pathogens, between baseline and 1 month. Similar frequencies of detection were detected at baseline in both groups, although a higher prevalence of *Porphyromonas gingivalis* was observed in the test group (53.8% versus 39.3%). After 1 month, all the evaluated pathogens showed some increase in the prevalence in the negative control group. Conversely, the test group showed minor changes for some pathogens, such as increases for *Parvimonas micra* and *Eikenella corrodens*, while there was a marked decrease for *Prevotella intermedia* (from 84.6 to 68%).

A higher variability was observed for counts and proportions of these selected pathogens, with a tendency to an increase in the proportions in the negative control group, in comparison with minimal changes or even decreases in the test group (e.g. for *P. gingivalis*, proportions decreased from 8.99 to 3.35%). No overgrowth of opportunistic species was detected in any group.

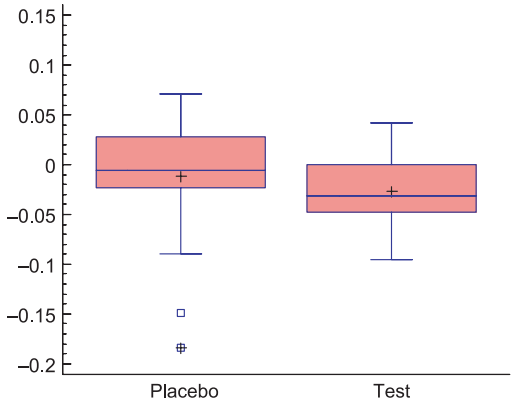

Fig. 3. Box & Whisker plot showing changes in the percentage of sites with 4–6 mm of probing pocket depth between baseline and 1 month, in each study group.

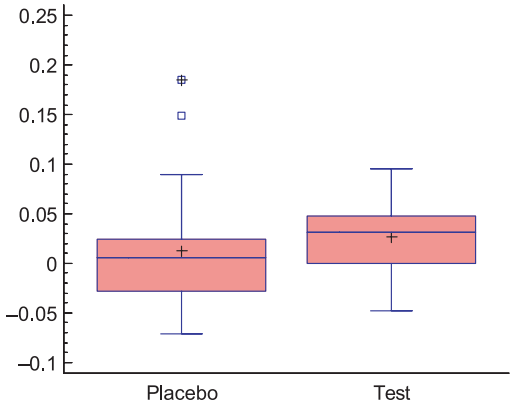

Fig. 4. Box & Whisker plot showing changes in the percentage of sites with 1–3 mm of probing pocket depth between baseline and 1 month, in each study group.

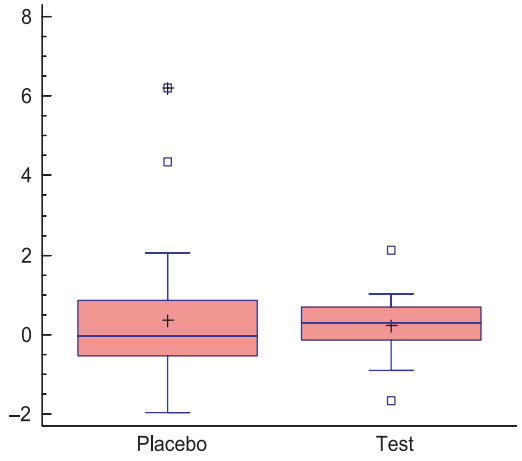

Fig. 5. Box & Whisker plot showing changes in the mean log of total bacterial counts, in colony forming units, between baseline and 1 month, in each study group.

Table 5. **Mean of pathogen counts and mean proportions of flora in positive site (proportions+) of different periodontal pathogens, per group and study visit**

		A.a.	*P.g.*	*P.i.*	*T.f.*	*P.m.*
Baseline						
Placebo	Mean counts	0	82 915	153 476	13 035	57 187
	Proportions (+)	na	11.49%	8.36%	3.73%	9.88%
Test	Mean counts	0	56 661	36 303	178	4747
	Proportions (+)	na	8.99%	4.24%	0.82%	3.95%
1 month						
Placebo	Mean counts	83	173 022	49 289	4011	17 746
	Proportions (+)	0.04%	14.47%	4.05%	6.23%	3.85%
Test	Mean counts	86	41 007	73 672	1056	12 276
	Proportions (+)	0.04%	3.35%	3.20%	5.26%	3.95%

A.a., Aggregatibacter actinomycetemcomitans; P.g., P. gingivalis; P.i., P. intermedia; T.f., Tannerella forsythia; P.m., P. micra; na, not available.

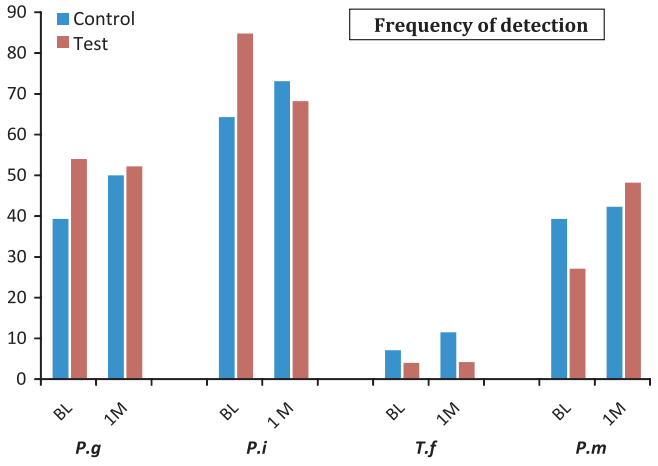

Fig. 6. Frequency of detection in percentage of different periodontal pathogens, per group and study visit at baseline (BL) and 1 month (1 M). *P.g., P. gingivalis; P.i., P. intermedia; T.f., T. forsythia; P.m., P. micra.*

The analysis of the questionnaire showed no self-reported side effects, such as taste discomfort, burning of the mouth, mucosal alterations, tongue de-papilatation and stain on teeth and/or tongue, which were not reported by any patient, except one patient in the test group who reported the occurrence of an aphthous lesion.

Stain intensity increased slightly in both groups. In the negative control group, the mean change of stain intensity from baseline to 1 month was 1.1 in the buccal aspect and 0.4 in the lingual aspect. In the test group, those values were 0.8 and 0.3, respectively. There were no statistically significant differences both for intergroup and for intragroup comparisons ($P > 0.05$). Similarly, when analysing the changes in stain area from baseline to 1 month, there was a slight increase in both groups, but differences between test and negative control groups were not statistically significant. In the control group, these changes were 0.7 and 1.1 for buccal and lingual areas and in the test group, 0.0 and 0.6, respectively. See Fig. 7.

Compliance was evaluated by measuring the remaining liquid in the returned bottles. This assessment was available from 16 test patients and 22 control patients. The mean amount of returned liquid was similar in both groups (236 ml in the control and 264 in the test group, with no significant differences between groups). Patients were provided with two 500-ml bottles and used approximately 75% of the mouth rinse in 4 weeks.

Discussion

The results of this investigation demonstrated some effects on plaque accumulation of the tested formulations, when used as an adjunct to unsupervised oral hygiene, but no effect was observed for gingivitis. The use of the test products (dentifrice and mouth rinse) showed this additional benefit by reducing plaque accumulation, as demonstrated by the statistically significant differences between the experimental and negative control groups at the lingual sites and substantiated

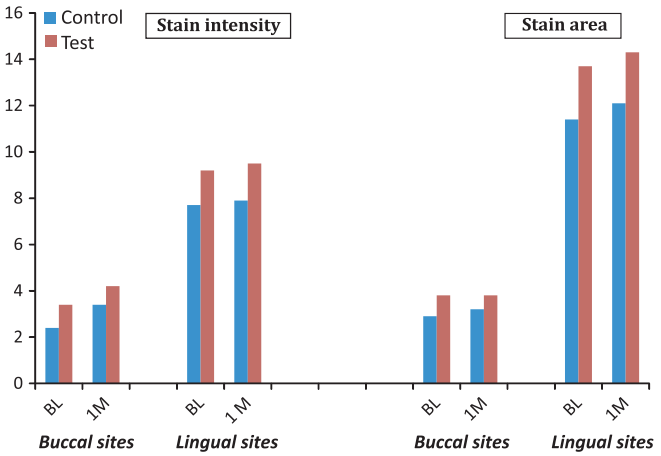

Fig. 7. Stain intensity and area expressed as mean at baseline (BL) and 1 month (1 M).

by the tendency to statistically significant reductions observed for the overall mean PlI ($P < 0.08$). The test group also demonstrated a significant reduction in gingivitis levels, although the intergroup differences did not reach significance owing to a similar gingivitis reduction in the placebo group.

Our results, showing a significant effect on plaque accumulation but not on gingivitis levels, are in agreement with a previous report using a 0.05% CPC mouth rinse in a similar study design (22). Regarding differences in the results of different tooth sites, to our knowledge, only one study (23) has shown different results at different tooth sites, as it failed to demonstrate the effects of CPC interdentally. It is also important to stress that the studies on CPC mouth rinses, which have demonstrated a significant effect in PlI when compared with a placebo, are all longer-term clinical trials using higher concentrations of CPC in different CPC formulations, whereas our results show efficacy in terms of plaque reduction over a short period by the use of 0.05% of CPC (13, 23–25). Other issue of discussion would be the clinical significance of the magnitude of the observed reductions in plaque. Overall, the test group demonstrated a 3.04% of reduction, versus 0.24% in the control group. The magnitude of the benefits could be considered then as limited. However, it is important to highlight the reduction in the PlI at the lingual surfaces that could be clinically interesting, as these sites are more difficult to brush: at lingual sites, control sites revealed an increase of 0.31% of plaque, versus a 3.62% of decrease in the test group. Other sites with a clearer benefit were non-proximal sites, because the magnitude of the decrease was 9.20% in test patients, versus 1.11% in control patients.

When assessing changes in gingival inflammation, GI was significantly reduced in both test and negative control groups, but there were no significant differences between them. There are only few studies that have reported significant differences in GI (13, 24, 25). However, all of them are longer-term studies, with formulations containing higher CPC concentrations (0.07% and 0.1%) and using different GIs. Conversely, studies using mouth rinses with 0.05% CPC have not detected significant differences, in agreement with the results of the present study (23, 26). There is a higher impact on gingivitis when CPC mouth rinses are used in long-term studies than in intermediate-length studies (6). However, 1-month studies are also important as they may inform on the efficacy to reduce short-term gingivitis (27). In addition, mouth rinses are also prescribed for short periods of time, so assessing their short-term effect could also be of interest and may have an impact on the efficacy. The lack of statistical differences between the test and the negative control groups could be related to the reduction in GI in the negative control group. In this type of study, with unsupervised toothbrushing, the Hawthorne effect is not minimized and may have a relevant effect on the results.

The impact of other active ingredients of the tested formulations, as zinc lactate, permethol and provitamine B5, may have added some effect, but there is no evidence that, in the low concentrations used, they can have some appreciable plaque inhibitory effect. Regarding the antimicrobial actions of the zinc, results have been contradictory and dependent on its concentration. Zinc alone may have some plaque inhibitory capacity at relatively high concentration (28) or in combination with other active agents such as triclosan (29) or hexetidine (30).

The microbiological results show that these agents, in both mouth rinse and toothpaste formulations, when used as adjuncts to mechanical plaque control, have a limited impact in terms of changes in subgingival bacterial counts. On the other hand, the results found a positive effect of the tested formulations on the frequencies of detection of some target pathogens, which was especially relevant for *P. intermedia* (decrease from 84.6 to 68% in the test group, versus an increase in the placebo group). In regard to the proportions of these pathogens in the subgingival miccrobiota, minor changes or even decreases in the test group (e.g. for *P. gingivalis*, proportions decreased from 8.99 to 3.35%) were observed. Even though the antibacterial activity of CPC has been demonstrated (8), the variability of the microbiological results and the small magnitude of the observed changes reflect a minor impact of the 0.05% CPC mouth rinse in the microbiological variables. Focusing on microbiological side effects, no overgrowth of opportunistic species was detected in any group.

There is a perception that the efficacy of antimicrobial compounds is positively correlated with increased tooth staining, and therefore, efficacy implicates likely negative aesthetic consequences resulting in a reduction of patient usage and thus compromising compliance. There are few studies that have evaluated staining with the use of CPC compounds. In fact, there is only one study reporting the staining of tongue and teeth with the use of 0.1% CPC (31), although this effect is probably due to the high concentration used. In this study, tooth staining was measured by intensity and area of staining by two calibrated examiners, with both groups having a similar percentage of smokers. There were no significant intra- and intergroup differences, and the use of the tested product did not have any impact on compliance because participants in both groups used their assigned products similarly during the 4-week study (75% usage). Moreover, the evaluation of other possible side effects, such as the presence of ulcerations, burning mouth sensation, discomfort in taste or sensitivity, did not demonstrate significant occurrence in the test group, which may indicate that the use of CPC compounds causes less side effects than other antiplaque agents such as chlorhexidine, which may indicate the use of this products for long-term as an adjunct to mechanical oral hygiene.

A recent systematic review on the efficacy of CPC-containing mouth rinses (6) reports heterogeneous results, with an overall small but significant additional benefit, when compared with a placebo rinse, in terms of reduction in plaque and gingival inflammation, when used as an adjunct to unsupervised oral hygiene. One of the factors responsible for these inconsistent results could be the different bioavailability of the active agents in the mouth rinse formulation. The FDA subcommittee recommends a bioavailability (drug available at site of action) of CPC ranging from 72 to 77%. The results from the present investigation show that this formulation with a low CPC concentration (0.05%) is readily bioavailable to penetrate

the biofilm and reduce plaque formation, without the typical side effects shown with the use of other antimicrobial compounds with proven antiplaque and antigingivitis efficacy.

Conclusions

The results of this investigation imply that the use of a dentifrice and a mouth rinse with 0.05% CPC, when used as an adjunct to unsupervised oral hygiene, may provide some benefits in reducing plaque accumulation in patients with gingivitis, with no relevant clinical or microbiological side effects. However, no effect on gingivitis was observed.

From a clinical perspective, the tested products may be recommended owing to the lack of adverse effects, and the evident clinical benefits, although they are restricted to plaque accumulation and no effect on gingivitis was observed. Longer-term studies may be performed to confirm the previous findings and to evaluate whether an effect on gingivitis could be observed if the period of follow-up is extended.

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