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Efficacy of a low-concentration chlorhexidine mouth rinse in non-compliant periodontitis patients attending a supportive periodontal care programme: a randomized clinical trial

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Abstract

Objective: To assess the clinical and microbiological efficacy of a 0.05% chlorhexidine and 0.05% cetyl-pyridinium chloride mouth rinse in supportive periodontal care (SPC) in patients with inadequate plaque control.

Material and Methods: The study was a randomized, double-blinded, placebo-controlled clinical trial in patients with moderate to severe chronic periodontitis under SPC with an inadequate plaque control (Turesky index >1). After supragingival prophylaxis and oral hygiene reinforcement, participants rinsed twice a day for 3 months with the test or placebo solutions, in addition to conventional hygiene. Primary clinical outcome variables included plaque and gingival indices. As secondary outcomes, periodontal and microbiological variables were studied. ANCOVA and χ^2 tests were used to compare the variables.

Results: Forty-seven patients (22 placebo and 25 test group) participated. After 3 months, plaque levels increased in the placebo group, while diminished in the test group ($p<0.001$). Similar effects were found for bleeding on probing. The other clinical parameters did not show significant differences. Microbiological variables demonstrated inter-group significant reductions in subgingival counts of *Fusobacterium nucleatum* and *Prevotella intermedia* and a decrease of the total bacterial counts in saliva.

Conclusions: The tested mouth rinse demonstrated efficacy in reducing plaque and gingivitis, as well as in decreasing the microbial load in saliva and gingival sulcus.

Key words: cetyl-pyridinium chloride; chlorhexidine; oral hygiene; periodontitis; supportive care

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Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

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It has been convincingly demonstrated that long-term stability of the clinical benefits obtained by periodontal therapy can only be achieved if a cause-related treatment is followed by effective supportive periodontal care (SPC) (Axelsson & Lindhe 1981, Becker et al. 1984). Within this SPC programme, it has also been demonstrated that self-performed plaque control is crucial to attain the best long-term results after periodontal therapy (Lindhe et al. 1984). As patient compliance with mechanical oral hygiene practices is not always as good as desired, chemical agents have been used to further improve plaque control and reduce gingivitis. Different antimicrobial agents have been studied

for their plaque inhibitory and antiplaque efficacy (Mandel 1988, Jorgensen & Slots 2001, Wu & Savitt 2002). From these studies, chlorhexidine (CHX) digluconate can be considered the gold standard for oral antiseptics due to its superior clinical and microbiological effects (Lang et al. 1988, Brecx et al. 1990, 1992).

This antibacterial activity of CHX is dosage dependent, with a threshold of 0.2% as the level over which no further benefits can be expected (Jenkins et al. 1994, Smith et al. 1995, Ernst et al. 1998). The downside of this antimicrobial compound is the appearance of undesirable side effects, mainly tooth staining, burning feeling and soft-tissue irritation. These side effects are also dosage dependent, being accentuated at concentrations above 0.1% (Smith et al. 1995). In order to reduce these side effects for long-term use of CHX, a reduction in the concentration of CHX (0.05%) has been proposed. To compensate the likely decrease in clinical efficacy, this mouth rinse has been reformulated with the addition of another antimicrobial agent, cetyl-pyridinium chloride (CPC). CPC is a quaternary ammonium compound, included in the group of cationic surface-active agents (Mandel 1988), that has demonstrated a moderate degree of efficacy as an antiplaque agent.

Changes in the formulation of oral hygiene products may, however, produce an impact on their activity, and therefore these ''improved'' formulations need to be evaluated in well-designed clinical studies (Herrera et al. 2003). Results from a short-term clinical trial (Santos et al. 2004) using this formulation containing 0.05% CHX, CPC and no alcohol demonstrated plaque-inhibitory activity and antibacterial efficacy in patients in SPC. The duration of this study was, however, only 15 days, and studies of longer duration are needed to assess the efficacy of oral hygiene products.

The aim of this investigation was to evaluate the clinical and microbiological efficacy of a new mouth rinse formulation (with low CHX concentration, without alcohol and with CPC), when used as an adjunctive method of mechanical oral hygiene for 3 months. The target population were patients under SPC, with non-optimal self-performance plaque control. In addition, clinical and microbiological adverse effects were monitored.

Material and Methods

Study population

Consecutive patients were selected in two centres (the Graduate Clinic of Periodontology at the University Complutense in Madrid, Spain, and the Department of Periodontology at the School of Dentistry in Leuven, Belgium) from their respective SPC programmes when fulfilling the following inclusion and exclusion criteria.

Inclusion criteria

- Adult patients, older than 18.
- Moderate to advanced chronic periodontitis (Armitage 1999).
- Basic periodontal treatment received in the previous 6 months.
- Turesky plaque index >1, at re-evaluation.
- Patients systemically healthy, and without relevant chronic medication intake.

Exclusion criteria

- Pregnant women or in lactation.
- Active periodontitis, with clear need of additional treatment [defined as having ≥ 2 sites per quadrant with probing pocket depth (PPD) $\geqslant 6$ mm].
- Known allergies to CHX or CPC.
- Systemic antibiotic intake in the previous month.
- Mouth rinse usage in the previous month.

All patients signed an Institutional Review Board-approved consent forms to participate in the study, after receiving detailed information about the purpose, the benefits and the possible hazards associated with the trial.

Experimental design

This study was designed as a randomized, parallel, dual-centre, double-blind, placebo-controlled, 3-month clinical trial.

During the screening visit, subjects were assessed for suitability to be included in the study by an oral examination and a medical and dental history. This screening visit occurred 1–6 months after receiving basic periodontal therapy, and if fulfilling the criteria and after accepting to participate by signing the IRB-approved informed consent, they were appointed for the baseline visit.

At baseline, an oral examination was carried out, assessing plaque accumulation, gingival inflammation (GI) and oral soft-tissue conditions. Moreover, microbiological samples were taken. Because of the non-optimal oral hygiene conditions of the patients, a supragingival scaling and a re-instrumentation of their residual periodontal pockets with an ultrasonic device were performed for, approximately, 1 h.

After this professional prophylaxis, all subjects received standardized oral hygiene re-instructions and were provided with a new toothbrush (Vitis Medio®, Dentaid, Barcelona, Spain), inter-dental brushes (Interprox Plus®, Dentaid) or dental floss (Vitis Seda Dental®, Dentaid), and a toothpaste containing sodium fluoride (FluorAid®, Dentaid). Besides, all subjects were asked to rinse twice daily, immediately after brushing during 30 s with 15 ml of the assigned product.

After 3 months, they were asked to return for an oral examination to record the same clinical parameters and to retrieve microbiological samples. Moreover, at this last visit the participant's compliance was assessed by measuring the remaining product from the returned mouth rinse bottles and by measuring their degree of satisfaction with the product's usage, by a brief interview. All patients then received a professional prophylaxis, and proceeded with their assigned SPC.

Treatments

An external agent randomized the treatments, by two computer-generated lists, one for each centre, by coding identical bottles with either test or placebo mouth rinses with consecutive numbers. Numbers were assigned to patients consecutively. Patients were stratified in two categories according to their tobacco habit as: non-smokers (including non-smokers and smokers of <10 cigarettes/day) and smokers of 10 or more cigarettes/day. Codes were not revealed until the study was finished. Both the examiners and the subjects were blinded to the content of the bottles. No attempt to blind examiners for tooth staining was made. The experimental mouth rinse formulation contained no alcohol and 0.05% CHX digluconate and 0.05% CPC as active ingredients, as well as

water, glycerin, propylene glycol, xylitol, peg-40 hydrogenated castor oil, sodium saccharin, potassium acesulphame, neohesperidine DC, aroma and C.I. 42090 (Perio-Aid Maintenance (R), Dentaid). The placebo rinse was identical, except that it lacked the active agents, CHX and CPC.

Clinical study

Two calibrated examiners in each centre carried out the oral examinations, being always the same examiner who assessed the outcome variables in the same patient at baseline and at the 3-month follow-up visits. The following clinical parameters (in sequential order) were recorded (before the re-instrumentation at baseline), at six sites per tooth in the entire mouth excluding the third molars:

- Degree of visual GI via the modified gingival index (Lobene et al. 1986).
- PPD and gingival recession, recorded to the nearest millimetre using a manual probe (Merrit B® probe, Hu-Friedy, Chicago, IL, USA). Clinical attachment levels (CAL) were calculated for each site by adding PPD and gingival recession.
- Bleeding on probing (BoP) evaluated 20 s after probing to the depth of the pockets.
- Plaque extension (PlI) after plaque disclosure with a 2% aqueous erythrosin solution. A cotton swab was submerged 10 s in the solution, and then applied to the tooth surfaces. After rinsing with water once, plaque deposits were assessed with the Quigley & Hein (1962) index modified by Turesky et al. (1970), with scores from 0 to 5.

The changes in PlI and GI between the baseline and final visit were considered as the primary outcome parameters.

Microbiological study

The following two samples (in sequential order) were collected at both baseline and 3-month visits:

- 1 ml of unstimulated saliva (representative for the microbial load in the oral cavity) (Umeda et al. 1998) was collected by asking the patient to move the tongue over the lips and cheeks, and spit the saliva content in a graduated glass container containing 4 ml of pre-reduced transport medium (RTF).
- Pooled subgingival sample: from each quadrant, the most accessible site with the deepest probing depth and bleeding was selected. Clinical variables were specifically recorded at these sites, such as the presence of plaque, bleeding on sampling, PPD, and gingival recession. Samples were taken with two consecutive sterile medium paper-points (Maillefer, Ballaigues, Switzerland) per site. Subgingival plaque was sampled after the removal of all supragingival plaque and debris (Wikstrom et al. 1991). Before sampling, the sites were isolated from saliva by applying cotton rolls and then gently dried with compressed air, in order to avoid contamination. The paper-points were kept in place for 10 s and were then transferred into a screw-capped vial containing 1.5 ml of RTF (Syed & Loesche 1972). Samples were transferred to the microbial laboratory within 2 h, where they were homogenized by vortexing for 30 s (Dahlen et al. 1990), and serially diluted in PBS.

Both samples were processed in a similar way at the laboratory. Aliquots of 0.1 ml were plated manually for the detection of *Aggregatibacter actinomycetemcomitans* on the specific medium Dentaid-1 (Alsina et al. 2001). These plates were incubated for 3 days in air with 5% CO_2 at 37°C. Suspected isolates were identified on the basis of colony morphology (small colony, 1 mm in diameter, with a dark border and a ''star'' or ''crossed cigars''-shaped inner structure) and positive catalase reaction. Sample dilutions were also plated onto a non-selective blood agar plate (Blood Agar Base II®, Oxoid, Basingstoke, England), supplemented with haemine (5 mg/l), menadione (1 mg/l) and 5% of sterile horse blood. After 7–14 days of anaerobic incubation (80% N_2, 10% CO_2 and 10% H_2), total counts and counts of representative colonies (those with colony morphologies compatible with target pathogens morphology) were performed in the most suitable plates, those harbouring between 30 and 300 colonies. Suspected colonies were further identified by microscopy, by studying gram-staining and enzyme activity (including *N*-acetyl-β-D-glucosaminidase, α-glucosidase, α-galactosidase, α-fucosidase, esculin, indole and trypsin-like activity). Counts were transformed in colony-forming units (CFU) per millilitre of the original sample. Total anaerobic counts were calculated, as well as counts of the periodontal pathogens detected (*A. actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Prevotella intermedia/nigrescens*, *Parvimonas micra*, *Campylobacter rectus* and *Fusobacterium nucleatum*). In addition to the quantitative microbiological data, the frequency of detection and proportions for each bacterial species were also calculated.

To assess microbiological adverse effects (overgrowth of super-infecting or opportunistic bacterial species, such as enterics), the presence of overgrowth of other colony types was monitored, especially in Dentaid-1 plates.

Adverse effects and compliance

At the final visit, different outcome variables were studied to assess the occurrence of adverse effects:

- A thorough examination of the oral mucosa was conducted for detecting any tissue reaction that could be attributed to product use.
- A focus interview with the patient assessing undesirable side effects such as tooth staining, tongue staining, burning feeling, changes in taste perceptions and oral dryness. The occurrence of these outcomes was recorded through a visual analogue scale. In addition, an interview was performed to record the patient's opinion of the product, including its taste, using a visual analogue scale. In this interview, the compliance with the use of the product was also evaluated.

Statistical analyses

A sample size calculation was performed based on the changes on plaque that occurred in a previous study (Santos et al. 2004), rendering a standard deviation of approximately 0.50 (0.55 in the test group and 0.38 in the placebo) for changes between baseline and 15 days. Considering a power of 80%, 18 patients needed to be included in each arm to detect a difference of 0.48. To compensate for drop outs, 22 patients were planned as the minimum sample.

An inter-examiner calibration was performed before the start of the study. Two patients (providing 156 sites) were evaluated by the researchers. The percentage of agreement was 69.9–88.5% for GI, 70.5–78.8% ($\leqslant 1$ mm) for PPD and 70.5–73.7% for BOP. The degree of agreement was considered as adequate.

For the analyses of the data, the patient was considered as the statistical unit. For each of the clinical outcome variables, the mean score per subject was calculated, both at baseline and at the 3-month visit. At baseline and at 3 months, differences between the test and placebo group were analysed by means of the Student *t*-test. Intra-group differences were assessed by means of a paired *t*-test.

For each primary outcome variable (PlI and GI), the components of variance were assessed by including in the model: treatment group, baseline value of the evaluated variable, other baseline values (PlI, GI, PPD), centre, examiner, gender, age and smoking. Then, an analysis of variance was carried out using the treatment as the factor and the baseline values of the evaluated variable as the covariate.

For the microbiological variables, bacterial counts (expressed as mean and standard deviation) were log transformed in order to achieve a normal distribution. The logs of zero values were considered as zero for convenience. Paired and unpaired *t*-test were used for intra-group (baseline *versus* 3 months) and inter-group (at baseline, at 3 months, and in changes at baseline–3 months) evaluations. Frequencies of detection were compared using the χ^2 test, either in the inter-group, at baseline and at 3 months, or in the intra-group. Proportions of flora were compared using the sign rank test (intra-group) or by the Wilcoxon test or the *t*-test (for non-normal or normal distribution, respectively) for inter-group assessment at baseline, at 3 months and in changes at baseline–3 months.

Results

Patients

Forty-seven patients were enrolled in the study, 36 in Madrid and 11 in Leuven, from April 2005 to January 2008 (Fig. 1). All participants attended both the baseline and the 3-month visits. No significant differences were detected between patients from both centres, and no centre-influence was observed in the results. Based on this, patients from both centres were pooled and analysed together.

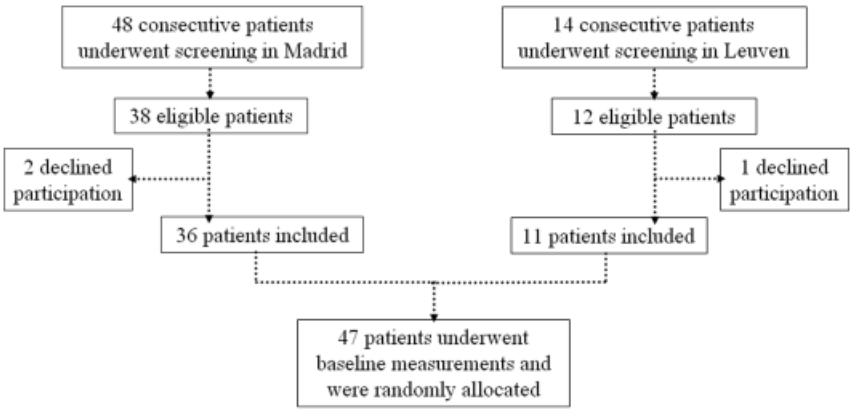

Fig. 1. Flowchart of patient inclusion.

Demographic characteristics are shown in Table 1. Twenty-two patients were assigned to the placebo group (mean age of 57, ranging 44–77, 14 females, five smokers) and 25 to the test group (mean age of 56, ranging 43–75, 12 females, seven smokers). No significant differences between groups were detected at baseline either in the demographic or the clinical variables (Table 2), with the exception of the percentage of BoP, which was significantly higher in the test (46.5% ± 18.9%) as compared with the placebo group (32.4% ± 14.7%).

Table 1. Demographic characteristics of both study groups

	Placebo	Test
Age		
Mean	56.7	55.8
SD	9.3	8.4
Maximum	77	75
Minimum	44	43
Gender		
Female	14	12
Male	8	13
n	22	25
Smokers		
No	17	18
Yes	5	7
Centre		
Madrid	17	19
Leuven	5	6

Clinical outcome variables

Baseline and 3-month values are shown in Table 2, and changes between baseline–3 months are shown in Table 3.

Plaque index

The PlI in both groups was almost identical at baseline (2.86 in the placebo group and 2.87 in the test group). Significant ($p<0.001$) higher values were detected after 3 months in the placebo group (3.03 *versus* 2.10, respectively). The inter-group differences in the changes between baseline and 3 months were also statistically significant ($p<0.001$), with an increase of 0.16 for the placebo group and a decrease of 0.64 for the test group. In the analysis of variance (ANOVA) model, the treatment showed the highest effect, while baseline PlI and gender were covariates. No influence of centre, examiner, age, smoking, baseline GI or baseline PPD was detected.

Gingival index

The GI in both groups was almost identical at baseline (0.96 in the placebo group and 0.99 in the test group). After 3 months, even though the test group showed lower scores than the control group (0.46 *versus* 0.56, respectively), the inter-group differences in GI were not statistically significant. Both groups showed statistically significant reductions in gingival inflammation between baseline and 3 months (Table 3), which were higher in the test group. However, these differences were not statistically significant. In the ANOVA model, no significant treatment effect was observed. Conversely, baseline PlI and baseline GI demonstrated an impact on the results. No influence of centre, examiner, age or smoking was detected.

Table 2. Mean values and standard deviation (SD) of different clinical variables at baseline and after 3 months

	Placebo		Test		*p* inter-group
	mean	SD	mean	SD	
Baseline					
Mean GI	0.96	0.92	0.99	0.78	NS
Mean PII	2.87	0.83	2.86	0.65	NS
Mean PPD	2.80	0.46	2.99	0.47	NS
Mean % of 1–3 mm pockets	81.79%	13.15%	74.20%	15.81%	NS
Mean percentage of 4–6 mm pockets	16.78%	11.74%	24.58%	15.26%	NS
Mean % of >6 mm pockets	0.85%	1.85%	0.72%	1.15%	NS
Mean BoP	32.42%	14.70%	46.52%	18.91%	0.007
Mean CAL	3.72	0.64	3.56	0.88	NS
Mean % of 1–3 mm CAL	50.47%	17.06%	54.95%	22.23%	NS
Mean % of 4–6 mm CAL	41.87%	13.74%	37.96%	18.27%	NS
Mean % of >6 mm CAL	7.10%	5.68%	6.69%	6.81%	NS
3 months					
Mean GI	0.56	0.41	0.46	0.27	NS
Mean PII	3.03	0.62	2.10	0.90	0.000
Mean PPD	2.71	0.39	2.80	0.45	NS
Mean % of 1–3 mm pockets	85.48%	9.52%	80.79%	13.61%	NS
Mean % of 4–6 mm pockets	13.50%	8.55%	18.11%	12.73%	NS
Mean % of >6 mm pockets	0.56%	1.07%	0.73%	1.21%	NS
Mean BoP	33.39%	17.79%	35.52%	16.92%	NS
Mean CAL	3.44	0.70	3.40	1.00	NS
Mean % of 1–3 mm CAL	57.49%	18.75%	58.45%	24.45%	NS
Mean % of 4–6 mm CAL	36.65%	16.47%	34.63%	18.22%	NS
Mean % of >6 mm CAL	5.40%	4.07%	6.46%	7.80%	NS

NS, not statistically significant ($p > 0.05$).
GI, gingival inflammation; CAL, clinical attachment level; BoP, bleeding on probing; PPD, probing pocket depth; PlI, plaque extension.

Table 3. Changes expressed as mean and standard deviation (SD) in clinical variables (baseline–3 months), including paired *t*-test intra-group *p* value (*p* intra) and unpaired *t*-test inter-group *p* value

	Placebo			Test			*p* inter-group
	mean	SD	*p* intra	mean	SD	*p* intra	
Gingival index	−0.40	0.70	0.019	−0.52	0.65	0.001	0.568
Plaque index	0.16	0.72	0.325	−0.76	0.64	0.000	0.000
Mean probing pocket depth	−0.09	0.32	0.206	−0.19	0.34	0.010	0.318
Mean % of 1–3 mm pockets	3.70%	6.97%	0.021	6.60%	10.21%	0.004	0.257
Mean % of 4–6 mm pockets	−3.28%	6.39%	0.025	−6.46%	10.07%	0.004	0.198
Mean % of >6 mm pockets	−0.29%	1.11%	0.239	0.00%	0.41%	0.955	0.255
Bleeding on probing	0.33%	18.50%	0.936	−11.00%	14.60%	0.001	0.029
Mean clinical attachment level	−0.27	0.38	0.004	−0.15	0.43	0.087	0.329
Mean % of 1–3 mm CAL	6.71%	10.75%	0.010	3.50%	10.17%	0.098	0.307
Mean % of 4–6 mm CAL	−4.52%	10.83%	0.070	−3.33%	8.56%	0.063	0.685
Mean % of >6 mm CAL	−2.08%	3.60%	0.016	−0.23%	5.05%	0.825	0.155

BoP

Changes in BoP are shown in Table 3. Baseline values of BoP were significantly higher ($p = 0.007$) in the test group. After 3 months, no differences between groups were detected due to a significant reduction in BoP in the test group and minor changes in the placebo group. The inter-group comparison of the changes baseline–3 months in BoP revealed statistically significant differences ($p = 0.029$).

PPD

Changes in PPDs are shown in Table 3. Mean PPD demonstrated minor reductions from baseline to 3 months. The reduction was only statistically significant in the test group. No inter-group differences were detected.

Frequency distributions of different pocket categories did not show significant differences between groups. Both groups showed significant changes between baseline and 3 months, with an increase in shallow pockets ($\leqslant 3$ mm), and in parallel a decrease in moderate pockets (4–6 mm). The magnitude of these changes was higher in the test group; however, no significant differences were detected.

CAL

Mean CAL showed minor reductions from baseline to 3 months. No differences were detected between groups (Table 3).

Frequency distributions of different CAL categories did not show significant differences between groups. Both groups showed changes between baseline and 3 months demonstrating attachment level gains, but no significant differences between the groups were detected.

Patient-centred variables

Only the answers provided by one of the centres (Madrid) were evaluated. Seventeen subjects from the control group and 19 from the test group completed the questionnaires (Table 4).

Two subjects reported to a 1-week episode of intraoral ulcers during the study period. Both of them were in the placebo group.

Tooth staining was reported by four patients in the control group (23.5%) and by 14 in the test group (73.7%). These differences showed a tendency towards statistical significance ($p = 0.07$). The same occurred for the burning feeling of the mouth, with four patients in the control group and 14 in the test group referring to this condition ($p = 0.08$).

No statistically significant differences were found between groups for any of the other patient-centred variables assessed (tongue staining, taste alterations and feeling of dryness).

When the taste of the product or the overall opinion was assessed, no inter-group differences were observed.

Microbiological outcome variables: subgingival samples

Microbiological outcome variables were only evaluated in one centre (Madrid). Out of the 17 control patients in Madrid, 17 subgingival samples were available at baseline, while 15 were processed after 3 months (one sample was lost due to technical problems and another sample was not taken). Out of the 19 test

patients in Madrid, 19 provided samples both for baseline and 3-month visit.

At baseline, the frequency of detection of the different pathogens (Table 5) was similar in both groups, although a significantly higher frequency of detection for *P. micra* was observed in the placebo group (64.7% *versus* 26.3%, $p = 0.05$). Minor microbiological changes occurred in both groups between the baseline and the 3-month visit, without any statistically significant difference between groups at 3 months, or between baseline and 3 months in any group.

Total bacterial counts, expressed as log CFU showed similar values at baseline and at 3 months, although a small reduction was observed during the study, which was higher for the test group (0.32 *versus* 0.21). No significant differences were, however, observed in any comparison (Tables 6a and 6b).

Specific bacterial counts for each pathogen were comparable at baseline, although higher amounts of *P. intermedia* were observed in the test group ($p = 0.04$) and higher amounts of *P. micra* were found in the placebo group ($p = 0.04$). After 3 months, a significant reduction was observed in the counts of *F. nucleatum* in the test group ($p = 0.05$). A tendency towards statistical significance ($p = 0.08$) was also observed in the reduction of *P. intermedia* in the test group, and in the increase in *T. forsythia* in the placebo group. Overall, minor changes were observed and no significant differences were detected at the 3-month visit (Tables 6a and 6b).

No overgrowth of opportunistic species was detected.

Table 4. Patient-centred outcomes: frequency distribution of patient perceptions registered in a questionnaire (values from 0 to 10 in a Visual Analogue Scale)

	Value 0	Values 1–4	Values 5–10
Tooth staining: χ^2, $p = 0.070$			
Placebo	13	3	1
Test	5	7	7
Tongue staining: χ^2, $p = 0.300$			
Placebo	13	3	1
Test	7	8	4
Burning feeling: χ^2, $p = 0.081$			
Placebo	13	4	0
Test	5	6	8
Changes in tasting: χ^2, $p = 0.226$			
Placebo	11	4	2
Test	8	4	7
Dryness: χ^2, $p = 0.588$			
Placebo	10	2	5
Test	8	4	7
Other: χ^2, $p = 0.375$			
Placebo	16	0	1
Test	14	0	4

	Value 0	Values 1–4	Values 5–7	Values 8–10
Taste like or dislike: χ^2, $p = 0.510$				
Placebo	5	1	4	6
Test	1	1	6	9
Overall: χ^2, $p = 0.203$				
Placebo	5	1	2	9
Test	1	0	6	10

Microbiological outcome variables: saliva samples

Out of the 17 control patients in Spain, 15 samples were available at baseline and at 3 months, as two samples were lost due to technical problems (one at baseline and one at three months), while no samples were taken from one patient at any visit. Out of the 19 test patients in Madrid, 19 samples were available at both baseline and 3 months in the test group.

The detection of *A. actinomycetemcomitans*, *T. forsythia*, *C. rectus*, *Eubacterium* sp. *Capnocytophaga* sp. and *E. corrodens* was low in saliva samples and these results are not presented.

The frequency of detection (Table 7) of the evaluated pathogens was similar at baseline, except for *P. gingivalis*, which showed a lower prevalence in the test group (5.3% *versus* 40%). An increase in *P. gingivalis* in the test group, and a decrease in *P. intermedia* in both groups, were the main changes after 3 months.

Total counts (Table 7) were comparable at baseline, and no changes were observed in the placebo group, while a significant reduction ($p = 0.02$) was observed in the test group, although no significant differences between groups were detected ($p = 0.13$).

Bacterial counts (Table 7) were similar at baseline, except for the lower

Table 5. Frequency of detection of different periodontal pathogens in subgingival samples

Visit	Group	*Aa*	*Pg*	*Pi*	*Tf*	*Pm*	*Cr*	*Fn*	*Eu*	*Cap*	*Ec*
Baseline	Placebo	17.6%	70.6%	88.2%	11.8%	64.7%	5.9%	100.0%	5.9%	11.8%	35.3%
	Test	15.8%	68.4%	100.0%	26.3%	26.3%	10.5%	100.0%	0.0%	15.8%	21.1%
	Inter-group χ^2	0.881	0.888	0.418	0.497	0.048	0.615	NA	0.955	0.727	0.562
3 months	Placebo	20.0%	80.0%	93.3%	26.7%	40.0%	26.7%	93.3%	0.0%	20.0%	26.7%
	Test	20.0%	75.0%	90.0%	25.0%	50.0%	20.0%	90.0%	10.0%	20.0%	15.0%
	Inter-group χ^2	1.000	0.727	0.727	0.911	0.807	0.954	0.727	0.599	1.000	0.669
Baseline–3 months	Placebo intra-group χ^2	0.865	0.838	0.622	0.533	0.297	0.259	0.949	0.340	0.879	0.886
	Test intra-group χ^2	0.732	0.920	0.491	0.925	0.234	0.707	0.491	0.491	0.732	0.940

Aa, Aggregatibacter actinomycetemcomitans; Pg, Porphyromonas gingivalis; Pi, Prevotella intermedia; Tf, Tannerella forsythia; Pm, Parvimonas micra; Cr, Campylobacter rectus; FN, Fusobacterium nucleatum; Eu, Eubacterium sp.; Cap, Capnocytophaga sp.; Ec, Eikenella corrodens; NA, not available.

Table 6a. Log of colony-forming units (CFU) and standard deviation (SD) of total anaerobic counts and counts of different periodontal pathogens in subgingival samples: baseline and 3 months

Visit	Group	Variable	Total CFU	*Aa*	*Pg*	*Pi*	*Tf*	*Pm*	*Cr*	*Fn*	*Eu*	*Cap*
Baseline	Placebo	Mean CFU	6.52	0.65	3.83	4.30	0.64	3.06	0.24	5.14	0.27	0.57
		SD CFU	0.47	1.49	2.66	1.75	1.80	2.38	1.00	0.48	1.10	1.60
	Test	Mean	6.73	0.60	4.00	5.30	1.33	1.33	0.55	5.23	0.00	0.66
		SD CFU	0.51	1.45	2.88	0.67	2.28	2.29	1.66	0.45	0.00	1.57
	Inter-group	*t*-test	0.214	0.915	0.850	**0.039**	0.318	**0.034**	0.495	0.576	0.332	0.868
3 months	Placebo	Mean	6.27	0.88	4.15	4.52	1.09	2.13	0.88	4.64	0.00	0.86
		SD CFU	0.75	1.80	2.47	1.45	2.18	2.58	1.76	1.38	0.00	1.71
	Test	Mean	6.33	0.68	4.06	4.45	1.36	2.11	0.96	4.43	0.51	0.80
		SD CFU	0.67	1.43	2.51	1.76	2.42	2.24	1.98	1.59	1.56	1.65
	Inter-group	*t*-test	0.803	0.733	0.914	0.911	0.741	0.987	0.904	0.694	0.163	0.921

Bold values signify $p < 0.05$.

Aa, Aggregatibacter actinomycetemcomitans; Pg, Porphyromonas gingivalis; Pi, Prevotella intermedia; Tf, Tannerella forsythia; Pm, Parvimonas micra; Cr, Campylobacter rectus; Fn, Fusobacterium nucleatum; Eu, Eubacterium sp.; Cap, Capnocytophaga sp.; Ec, Eikenella corrodens; NA, not available.

Table 6b. Log of colony-forming units (CFU) and standard deviation (SD) of total anaerobic counts and counts of different periodontal pathogens in subgingival samples: changes between baseline and 3 months

Visit	Group	Variable	Total CFU	*Aa*	*Pg*	*Pi*	*Tf*	*Pm*	*Cr*	*Fn*	*Eu*	*Cap*
Baseline–3 months	Placebo	Mean CFU	0.21	0.00	− 0.19	0.10	0.00	0.07	0.00	0.13	0.00	0.00
		SD CFU	0.66	0.01	0.50	0.68	0.00	0.31	0.00	0.40	0.00	0.00
	Test	mean CFU	0.32	− 0.05	0.23	0.35	− 0.09	0.18	0.02	0.27	0.00	0.04
		SD CFU	0.87	0.24	1.02	0.99	0.26	0.55	0.07	0.71	0.00	0.16
	Inter-group	*t*-test	0.687	0.313	0.130	0.383	0.165	0.476	0.331	0.481	NA	0.331
	Placebo	*t*-test	0.250	0.444	0.292	0.987	0.083	0.310	0.336	0.194	0.336	0.814
	Test	*t*-test	0.125	0.967	0.764	0.080	0.881	0.332	0.398	**0.048**	0.163	0.992

Bold value signifies $p < 0.05$.

Aa, Aggregatibacter actinomycetemcomitans; Pg, Porphyromonas gingivalis; Pi, Prevotella intermedia; Tf, Tannerella forsythia; Pm, Parvimonas micra; Cr, Campylobacter rectus; Fn, Fusobacterium nucleatum; Eu, Eubacterium sp.; Cap, Capnocytophaga sp.; Ec, Eikenella corrodens; NA, not available.

Table 7. Frequency of detection, log of colony-forming units (CFU) and standard deviation (SD) of total anaerobic counts and counts of different periodontal pathogens in saliva samples

Visit	Group	Variable	Total Counts	*P. gingivalis*	*P. intermedia*	*P. micra*	*F. nucleatum*
Baseline	Placebo	Frequency of detection		40.0%	73.3%	20.0%	80.0%
		Mean log CFU	7.25	2.16	3.75	1.05	4.70
		SD	0.56	2.79	2.45	2.23	2.51
	Test	Frequency of detection		5.3%	73.7%	15.8%	84.2%
		Mean log CFU	7.28	0.25	4.04	0.93	5.01
		SD	0.61	1.11	2.53	2.21	2.31
3 months	Placebo	Frequency of detection		26.7%	46.7%	6.7%	93.3%
		Mean log CFU	7.24	1.45	2.41	0.32	5.37
		SD	0.66	2.51	2.69	1.24	1.65
	Test	Frequency of detection		42.1%	52.6%	15.8%	89.5%
		Mean log CFU	6.86	2.02	2.65	0.72	4.46
		SD	0.63	2.48	2.63	1.73	1.68
Baseline–3 months	Placebo	Mean log CFU	0.00	0.05	− 0.03	− 0.07	0.14
		SD	0.82	0.39	0.51	0.27	0.79
	Test	Mean log CFU	0.43	− 0.01	0.18	0.07	0.59
		SD	0.68	0.05	0.39	0.28	0.62
	Inter-group	*t*-test	0.13	0.55	0.21	0.17	0.09
	Placebo	Intra-group-*t*-test	1.00	0.32	0.34	0.21	0.28
	Test	Intra-group-*t*-test	0.02	0.00	0.13	0.43	0.45

amounts of *P. gingivalis* in the test group ($p = 0.02$) mentioned previously. After 3 months, all counts decreased, except a significant increase in *P. gingivalis* in the saliva ($p = 0.005$). The decrease of *F. nucleatum* counts in the test group demonstrated a tendency towards statistical significance, as compared with the increase in the placebo group ($p = 0.09$).

No over growth of opportunistic species (super-infecting or other opportu-

nistic bacterial species, such as enterics) was observed in any sample.

Discussion

The results from this 3-month randomized clinical trial have shown that a low CHX concentration mouth rinse combined with CPC offered an adjunctive effect by significantly reducing plaque and BoP in patients with an inadequate self-performance plaque control during SPC. These reductions in plaque and bleeding were paralleled with a reduction in subgingival counts of *F. nucleatum* and *P. intermedia* and with a decrease of total bacterial counts in saliva.

A first important element to consider is patient selection. Non-compliant patients with inadequate oral hygiene procedures were recruited for this trial. The extent to which a patient's behaviour coincides with medical advice is commonly referred to as adherence or compliance. Success in periodontal treatment is highly dependent upon the ability and willingness of the patient to maintain good oral hygiene and the degree of compliance with supportive periodontal treatment schedules. Most studies on periodontal maintenance show low levels of compliance with supportive therapy, varying from 11% to 45% (Wilson et al. 1984, Mendoza et al. 1991, Demetriou et al. 1995, Demirel & Efeodlu 1995, Novaes et al. 1996, Wilson 1996, Novaes & Novaes 1999, 2001, Konig et al. 2001). It is, however not clear, why patients who accept referral to a specialist clinic and undergo periodontal treatment, do not comply with the recommended maintenance therapy (Fardal 2006). Even when patients are interviewed shortly after oral hygiene instructions, they have shown high levels of non-compliance (Strack et al. 1980, Boyer & Nikias 1983, Johansson et al. 1984).

To overcome this problem and thus to enhance patients' home-care plaque control, oral antiseptic products have been recommended, both in toothpaste and mouth rinse formulations (Jenkins et al. 1994, Rosling et al. 1997, Gunsolley 2006, Ciancio 2007). These products have demonstrated their efficacy in reducing plaque and gingivitis in non-compliant patients, but they have also shown to cause side effects like tooth staining and taste alterations with their long-term use (Jenkins et al. 1994, Rosling et al. 1997, Gunsolley 2006, Ciancio 2007). With the aim of reducing these side effects, the use of low-dose CHX (Jenkins et al. 1994) or the combination of two or more oral antiseptics in home-care products have been proposed (Roldan et al. 2003, Winkel et al. 2003).

In the present study, the tested mouth rinse achieved reductions in the levels of plaque that were statistically significant both in the intra-group evaluation and in the evaluation of the plaque-level changes between groups. Similar outcomes have been reported in another study using the same product, although it was short-term (2-week follow-up) (Santos et al. 2004). In spite of this significant effect on plaque, no significant effect was detected in the gingival index. Different reasons could explain this fact. It has been suggested that the initial *Hawthorne effect* resulting in improved clinical outcomes in both groups, due to increase in motivation and compliance within the first 3 months, can mask possible differences in oral hygiene products (Mauriello et al. 1987, Graves et al. 1989). In fact, a significant reduction in the gingival index ($p = 0.001$) was observed in the test group, but the placebo group also showed a significant reduction ($p = 0.02$). Another possible explanation could be the inherent limitations of the gingival index to detect small differences in gingival inflammatory changes. This is clearly shown in this study since significant changes in gingival inflammation were evidenced by a more objective diagnostic method, BoP (Chaves et al. 1993, Barnett 1996). However, the results in BoP should be interpreted with caution, as statistically significant differences were found at baseline, with higher levels in the test group.

Changes in the secondary clinical variables were of small magnitude. The test group, however, demonstrated significant improvements in mean PPD, and in both groups there was a shift in the frequency distribution of pocket depth categories, towards shallower probing depths. Changes in these clinical variables were not statistically significant when both groups were compared, as reported previously with the short-term use of the same product (Santos et al. 2004).

The microbiological impact of the mouth rinse used was observed at the two oral niches sampled. At the subgingival niche, the use of the test rinse diminished subgingival counts of *F. nucleatum* and *P. intermedia*, and decreased the proportions of *P. gingivalis* and the counts of *T. forsythia*, without eliciting any adverse microbiological side effects. At the salivary niche, the test product also demonstrated an antimicrobial effect, as proved by the significant reduction in total counts and in the proportions and counts of *F. nucleatum*. However, an increase in the detection of *P. gingivalis* was also observed, which can be explained by the reduction in the total counts that makes easier the detection of *P. gingivalis*, even if a real increase in their counts did not occur. Similar results have been previously reported at the subgingival niche with the same product in a 15-day study, which found a significant reduction in the subgingival bacterial load together with a reduction in the levels of *P. gingivalis* (Santos et al. 2004). In addition, other reports have found an impact of oral hygiene products on the subgingival microflora, such as toothpastes with triclosan/copolimer (Rosling et al. 1997), and mouth rinses with CHX, CPC and zinc lactate (Roldan et al. 2003).

The tested product has been developed for its long-term use in periodontal patients under supportive care. For this purpose, firstly, the CHX concentration has been reduced to 0.05%, which may decrease the known adverse effects of this agent as compared with 0.12–0.2% concentrations. Although in this study a positive control was not used, the tested product produced more staining than the placebo formulation, as reported by the patients in the questionnaire. This finding was already seen after 2 weeks of use, in terms of the stain intensity evaluated by a clinician, as reported in a previous study (Santos et al. 2004). Secondly, no alcohol was included in the composition. The inclusion of alcohol in mouth rinses is still a controversial topic, due to the possible health risk associated with long-term use of alcohol-containing products (oral cancer, mucositis, etc.) (Winn et al. 1991, Shapiro et al. 1996). The presence of alcohol however, may have an important role in a formulation by enhancing the maintenance of the active ingredient bio-disposal and by avoiding, its contamination. In this study, as the clinical efficacy of the tested product was demonstrated, one could speculate that the possible lower plaque inhibitory and antiplaque efficacy, by reducing the CHX concentration and eliminating the alcohol, was compensated by adding

CPC to the formulation. It has been previously demonstrated that the addition of CPC to a CHX formulation can compensate the lack of alcohol in 0.12% CHX formulations and even improved its *in vivo* and *in vitro* activity (Quirynen et al. 2001, Herrera et al. 2003). Another potential side effect of the long-term use of an antimicrobial mouth rinse could be the increase in bacterial resistance. In the present research, no overgrowth of opportunistic species was detected, but additional long-term studies are needed to assess whether the use of low-dose CHX formulations may be associated with changes in bacterial resistance.

With regard to the efficacy of low CHX concentrations, although the plaque inhibition efficacy of CHX is dose-dependent and low-concentration rinses may be less effective, they still may be considered as adjuncts to oral hygiene (Jenkins et al. 1994). Previous investigations using a mouth rinse with 0.05% CHX, formulated to treat oral halitosis (also containing 0.05% CPC, 0.14% zinc lactate and no alcohol; Halita®, Dentaid), have demonstrated both efficacy in reducing halitosis (Winkel et al. 2003), as well as modifying the microbiological oral environment, including the subgingival microflora (Roldan et al. 2003).

Conclusion

The test mouth rinse demonstrated efficacy in reducing plaque levels and BoP scores, as well as a microbiological impact at both the salivary and the subgingival niches. These effects may improve the clinical conditions of treated periodontitis patients with an inadequate mechanical plaque control.

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Clinical Relevance

Scientific rationale for study: The establishment of efficient plaque control by proper oral hygiene measures is an essential prerequisite for the successful prevention of periodontal disease. Chemical plaque control may be useful, especially in patients with less than optimal mechanical plaque control. However, the mere inclusion of a known active agent does not assure that any new product is effective, and thus clinical studies are needed to assess their efficacy.

Principal findings: The use of the tested mouth rinse demonstrated significant reductions of plaque and BoP, when compared with the placebo. An impact on microbiological parameters in both plaque and saliva was also detected.

Practical implications: A mouth rinse with a low concentration of CHX (0.05%), combined with CPC may be useful during SPC in the prevention of gingivitis in periodontitis patients without a proper self-plaque control.