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A randomized clinical trial on the short-term clinical and microbiological effects of the adjunctive use of a 0.05% chlorhexidine mouth rinse for patients in supportive periodontal care

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Abstract

Objective: To evaluate the clinical and microbiological activity of a new mouth rinse formulation, used as an adjunct to oral hygiene, for patients in supportive periodontal care.

Patients and Methods: This was a randomized, placebo-controlled clinical trial with two groups: test group, rinsing twice per day with the test product (with 0.05% chlorhexidine and 0.05% cetylpyridinium chloride); and control group, rinsing with a placebo. Treated chronic periodontitis patients were included, and two visits were rendered, baseline, and after 15 days. Clinical outcome variables included plaque and gingival indices, and probing pocket depth. Subgingival samples were processed by culturing. Patient-based variables and adverse effects were also assessed. Outcome variables were compared by *t*-test, χ^2, and Mann–Whitney test.

Results: The results belonged to 33 patients. Plaque and gingival indices, and the log of bacterial total counts were reduced in the test group ($p \leqslant 0.01$), but differences between groups were only statistically significant ($p < 0.05$) for plaque and bacterial counts. A significant reduction in the proportions of flora ($p < 0.05$) and frequency of detection ($p = 0.01$) of *Porphyromonas gingivalis* was observed in the test group.

Conclusions: The newly formulated mouth rinse demonstrated short-term plaque-inhibitory activity. This was associated with a reduction in the total load of anaerobic subgingival microflora.

Key words: cetylpyridinium chloride; chlorhexidine; microbiology; mouth rinses; oral hygiene product; RCT; supportive periodontal care

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Based on the evident limitations of mechanical plaque control, there has been a broad search for many years on chemical agents that could supplement patient-dependent mechanical plaque control (Mandel 1988). In this manner, both mechanical and chemical antimicrobial intervention becomes the basis of both primary and secondary prevention of periodontal diseases.

In secondary prevention, successful supportive periodontal care depends upon the ability of oral health professionals to treat periodontal infections successfully, and on the patient compliance with the prescribed follow-up care (Jorgensen & Slots 2001). Since patient compliance is not always as

good as desired, chemical agents can help by further improving the control of plaque and gingivitis. Several 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 has clearly received the greatest attention due to its superior clinical and microbiological effects (Lang & Brecx 1986, Lang et al. 1988). Cetylpyridinium chloride (CPC) is a quaternary ammonium compound included in the group of cationic surface-active agents (Mandel 1988); it has demonstrated a moderate degree of efficacy as an antiplaque agent, but this efficacy is limited by the rapidity by which they are desorbed from oral tissue sites (Bonesvoll & Gjermo 1978).

When used as a mouth rinse, CHX (at 0.2% or 0.12%) has evidenced the appearance of undesirable side effects, such as staining, burning feeling, and soft-tissue irritation. Their use at these concentrations, or with more frequency, increases their clinical efficacy, and also accentuates these undesirable side effects.

In order to reduce these side effects and thus allowing its long-term use, it has been hypothesized that the reduction in the concentration of CHX (0.05%) should decrease their incidence and severity. However, to avoid a decrease in its clinical efficacy, the mouth rinse composition needed to be reformulated, such as by adding an additional antimicrobial agent, CPC. Moreover, by eliminating the presence of alcohol in the formulation, a safer long-term use may be achieved. These hypotheses have to be evaluated in clinical studies, since relevant changes in the formulation of oral hygiene products can have an important impact on their activity (Herrera et al. 2003).

The aim of the present study was to evaluate the short-term clinical and microbiological efficacy of a new mouth rinse formulation, with a low CHX concentration (0.05%), and CPC, used as an adjunctive method of oral hygiene, for patients in supportive periodontal care.

Patients and Methods

Study population

Consecutive patients were selected, among those attending the Graduate Clinic of Periodontology at the Universidad Complutense in Madrid, Spain, for supportive periodontal care, and fulfilling the following inclusion and exclusion criteria.

Inclusion criteria

- Adult patients, older than 18.
- Patients with chronic periodontitis, treated by means of scaling and root planning and/or periodontal surgery, and in supportive periodontal care for at least 1 year.
- A minimum number of 16 teeth, after excluding third molars.
- Patients systemically healthy, and without relevant chronic medication intake.

Exclusion criteria

- Pregnant women or in lactation.
- Professional dental cleaning in the previous month.
- Systemic antibiotic intake in the previous month.
- Periodontal treatment in the previous year.
- More than three pockets 4 mm or more deep.
- Frequent use of anti-inflammatory drugs, or drugs associated to xerostomia.
- Patients with removable prosthesis, or orthodontic appliances.

Additionally, other habits, such as smoking, were recorded by a directed interview, as well as any relevant systemic condition or medication intake.

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

Experimental design

This paper describes the short-term clinical and microbiological results of a randomized, double-blind, prospective, placebo-controlled, parallel group comparison clinical trial.

During the screening visit, subjects were assessed for suitability to be included in the study. At baseline, an oral examination was carried out, assessing plaque accumulation, gingival inflammation, and oral soft-tissue conditions. Oral photographs were obtained in order to assess for tooth staining. In addition, microbiological samples were taken.

All subjects were asked to continue their mechanical oral hygiene habits in their usual manner (no attempt was made to establish whether patients previously used power-driven toothbrushes), and to rinse immediately after brushing during 1 min with 15 ml of the assigned product, twice daily. A new toothbrush and toothpaste (containing 0.553 g of sodium fluoride) were provided to all subjects, and no additional oral hygiene instruction was provided.

The study lasted 2 weeks and subjects were asked to return for an oral examination where the same clinical parameters were recorded and microbial samples were taken. Moreover, at this visit the participant's compliance was assessed by measuring the remaining product after returning the bottle of mouth rinse, and their degree of satisfaction by means of a brief interview. After this visit, all patients received a professional prophylaxis, and proceeded with their assigned supportive periodontal care.

Treatments

An external agent randomized treatments, by coding identical bottles, with either test or placebo mouth rinse (following a computer-generated randomization list), with consecutive numbers. No attempt at balancing for smoking or other factors was possible. Codes were not revealed until the study was finished. Both the examiner and the subjects were blinded to the content of the bottles. The experimental mouth rinse formulation contained no alcohol and 0.05% CHX digluconate and 0.05% CPC as active ingredients (Perio-Aid Mantenimiento®, Dentaid, Barcelona, Spain). The placebo mouth rinse was identical, except that it lacked the active ingredients.

Clinical study

One calibrated examiner carried out the oral examinations. Teeth with cervical restorations, non-adjusted margins of fixed prosthesis, or subgingival restorations, as well as third molars, were excluded from assessment. The following clinical parameters (in sequential order) were recorded, at six sites per tooth:

Plaque indices: the dichotomous index (O'Leary et al. 1972), and the Quigley & Hein (1962) index, modified by Turesky et al. (1970), were visually evaluated.

Gingival indices: Muhlemann & Son (1971) and Loe & Silness (1963) were carried out.

Probing pocket depth and gingival recession: recorded to the nearest 0.5 mm using a manual probe.

Adverse effects and compliance

At the final visit, different examinations were conducted to assess the occurrence of adverse effects:

Tooth staining: standardized digital photographs (all taken by the same researcher, under the same conditions, with fixed flash, same size, and at an angle of 90°) of the buccal surfaces of the six mandibular anterior teeth were graded for dental stain (Sanz et al. 1994). A single numerical grade (0 = no staining to 6 = heavy staining) was assigned to reflect the overall stain score. Secondly, each separate tooth was graded for stain intensity on a 0–4 scale (0 = no staining to 4 = very dark stain), and coverage on a 0–6 scale (0 = no staining to 6 = more than 30% of coverage).

Oral soft-tissue health: a thorough examination of the oral mucosa was conducted in order to detect any tissue reaction that could possibly be attributed to product use.

Interview: side effects such as pain, sensitivity, change in the taste perception, and concomitant intake of medications were recorded. Compliance was evaluated by interviewing the subjects and by measuring the return product from the bottles.

Microbiological study

At baseline, and at 15 days, pooled samples of subgingival plaque were taken. 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, probing pocket depth, and gingival recession. Samples were taken with two 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). Prior to sampling, the sites where isolated from saliva by applying cotton rolls and then gently dried with compressed air, in order to avoid contamination (van der Velden et al. 1986). The paper-points were kept in place for 10 s and were then transferred into a screw-capped vial, containing 1.5 ml of pre-reduced transport medium, RTF (Syed & Loesche 1972). Samples were transferred to the laboratory within 2 h, where they were homogenized by vortexing for 30 s (Dahlen et al. 1990), and serially diluted in PBS. Adequate aliquots were plated on blood-agar (with hemin and menadion) medium, and anaerobically incubated for 15 days. Identification of bacterial species was based primarily on colony morphology, and then confirmed using different standard biochemical tests. The total counts and presence and number of different periodontal pathogens (*Porphyromonas gingivalis*, *Prevotella intermedia/nigrescens*, *Bacteroides forsythus*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Peptostreptococcus micros*) were obtained. An additional selective medium, Dentaid-1 (Alsina et al. 2001), was used for isolation of *Actinobacillus actinomycetemcomitans*.

Statistical analyses

Plaque and gingival indices were averaged by patient and then by group and visit. After assessing whether they fitted a normal distribution (skewness and kurtosis), and whether significant differences existed between variances (*F*-test), baseline and 2-week results were compared by paired *t*-test (intragroup), and changes between visits were compared by unpaired *t*-test (intergroup).

Probing pocket depth was evaluated in a similar way, but a Mann–Whitney test was applied for intergroup comparison.

The total colony-forming units (CFU) were averaged by group and visit, and log-transformed to fit a normal distribution. A *t*-test was employed, as described above.

The frequency of detection of distinct pathogens was compared intragroup by a χ^2 test. Differences in percentage within the total flora were compared by a Mann–Whitney test (intergroup), or a signed-rank test (intragroup).

Differences in staining and other patient-based variables were compared by means of a Mann–Whitney test.

To assess the possible influence of other factors, such as smoking, sex, age, and baseline levels of the measured variables, on the study outcome variables, an analysis of co-variance was used, considering these likely factors as the covariate. Only the baseline levels of the measured variables demonstrated a limited influence on the outcome.

Results

Study population

Thirty three patients were included, 17 in the test group, and 16 in the control group.

Their distribution with respect to gender, tobacco smoking, and age, in both groups, are shown in Table 1. No statistical significant differences were detected ($p = 0.75$).

Clinical results

One patient (placebo group) did not return for the final evaluation. Four additional test patients and three in the control group suffered a partial lack of clinical data. Clinical results belonged to 16 test patients and 15 control patients, with 14 pairs of results (baseline–final) in the test group and 13 in the control group.

Intragroup results are shown in Table 2, and intergroup results in Table 3.

Plaque indices

The results from plaque indices are shown in Fig. 1.

For the Turesky/Quigley–Hein plaque index, in the test group, a statistically significant ($p = 0.005$) decrease was obtained, representing a 40.8% reduction. The control group showed minor reductions. When the changes in plaque were compared between both groups, differences were statistically significant ($p = 0.023$).

For the dichotomous plaque index, in the test group, a statistically significant ($p < 0.001$) decrease was observed, representing a 38.5% reduction. The control group showed minor changes. When the changes in plaque were compared between both groups, differences were statistically significant ($p = 0.007$).

Gingival indices

The results from gingival indices are shown in Fig. 2.

Table 1. Description of the study population regarding age, sex, and smoking habit

	Test	Placebo
Age		
mean	46.6	45.8
SD	5.7	8.6
range	35–63	36–57
Sex		
male	5	7
female	12	9
total *n*	17	16
Smoking habit		
non-smoker	9	7
smoker > 10 cig/day	4	6
former smoker	4	3

SD, standard deviation.

Table 2. Intragroup evaluation of different outcome variables

	Test					Placebo				
	baseline		final		*p* value	baseline		final		*p* value
	mean	SD	mean	SD		mean	SD	mean	SD	
Full-mouth plaque indices										
dichotomous	0.63	0.18	0.39	0.23	<0.001	0.64	0.18	0.57	0.24	NS
Turesky	1.19	0.64	0.71	0.54	0.005	1.23	0.62	1.19	0.65	NS
Full-mouth gingival indices										
Silness & Löe	0.81	0.39	0.57	0.29	0.009	0.96	0.42	0.73	0.45	NS
Mühlemann & Son	0.71	0.32	0.45	0.3	0.015	0.73	0.42	0.63	0.49	NS
Full-mouth PPD	2.24	0.24	2.18	0.20	NS	2.38	0.25	2.30	0.25	NS
Sampled sites										
% plaque index	66.1	34.8	30.3	31.1	<0.001	66.7	38.6	61.7	33.9	NS
% bleeding on sampling	85.0	24.6	58.3	33.6	0.001	75.0	35.3	63.3	36.4	NS
PPD	2.68	0.68	2.56	0.53	NS	3.06	0.95	2.90	0.99	NS
Total bacterial counts										
anaerobic log-CFU	5.97	0.95	5.32	0.88	0.015	5.88	0.82	6.12	0.63	NS

SD, standard deviation; CFU, colony-forming units.

Table 3. Intergroup evaluation of different outcome variables

	Test		Placebo		*p* value
	mean*	SD	mean	SD	
Full-mouth plaque indices					
dichotomous	0.24	0.17	0.06	0.14	0.007
Turesky	0.49	0.55	0.04	0.38	0.023
Full-mouth gingival indices					
Silness & Löe	0.24	0.29	0.22	0.45	NS
Mühlemann & Son	0.25	0.34	0.09	0.45	NS
Full-mouth PPD	0.05	0.10	0.08	0.22	NS
Sampled sites					
% plaque index	35.7	0.29	5.0	34.3	0.01
% bleeding on sampling	26.6	25.8	11.6	56.6	NS
PPD	0.12	0.56	0.15	0.24	NS
Total bacterial counts					
anaerobic log-CFU	0.65	0.68	−0.24	0.89	0.01

SD, standard deviation, CFU, colony-forming units.
*Mean difference between baseline and final values. Positive means decrease.

For the Löe–Silness gingival index, a statistically significant ($p<0.009$) reduction was observed in the test group, representing a 29.4% decrease. The control group also showed a minor reduction, but not reaching significance. In the comparison between groups, differences were not statistically significant.

For the Muhlemann–Son gingival index, a statistically significant ($p<0.015$) reduction was observed in the test group, representing a 36% reduction. The control group also showed reductions, but without reaching significance. When comparing both groups, differences were not statistically significant.

Probing pocket depth

Minor changes were observed with respect to probing depths. Small reductions were detected in both groups, but none reaching statistical significance. The same was true regarding gingival recession and clinical attachment levels.

Clinical variables at sampled sites

The intragroup results are shown in Table 2, and intergroup results in Table 3.

Dichotomous plaque index

A significant reduction ($p<0.001$) was detected in the test group, while minor changes were observed in control patients. Significant differences were observed when both groups were compared ($p=0.015$).

Bleeding on sampling

A significant ($p=0.001$) reduction was recorded in the test group, while minor changes were observed in control patients. The intergroup comparison did not demonstrate significant differences.

Probing pocket depth

Minor non-significant changes were detected in both groups. The same was true for recession and clinical attachment levels.

Microbiological results

Microbiological results belonged to 32 patients, 17 in the test group and 15 in the control group. Owing to technical problems, some samples could not be processed, and therefore, the samples available for evaluation were 14 at baseline and 16 at final evaluation (13 pairs of results) for the test group, and 13 and 14, respectively (12 pairs), for the control group.

Total anaerobic CFU

The results from total bacterial counts are shown in Tables 2 and 3, and in Fig. 3.

The total counts expressed in log-CFU were significantly reduced ($p=0.015$) in the test group. Conversely, almost no changes were observed in the control group. Intergroup comparison detected significant differences ($p=0.017$) between the changes in the test and control groups.

Porphyromonas gingivalis

A significant ($p=0.011$) reduction in the frequency of detection of this bacterial species was observed in the

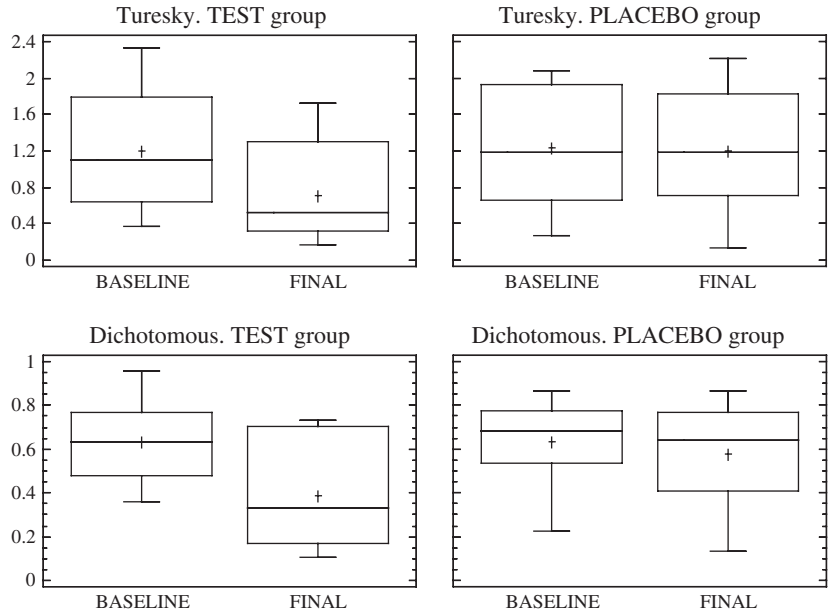

Fig. 1. Box and whisker plots of plaque indices for test and placebo groups, at baseline and final visits.

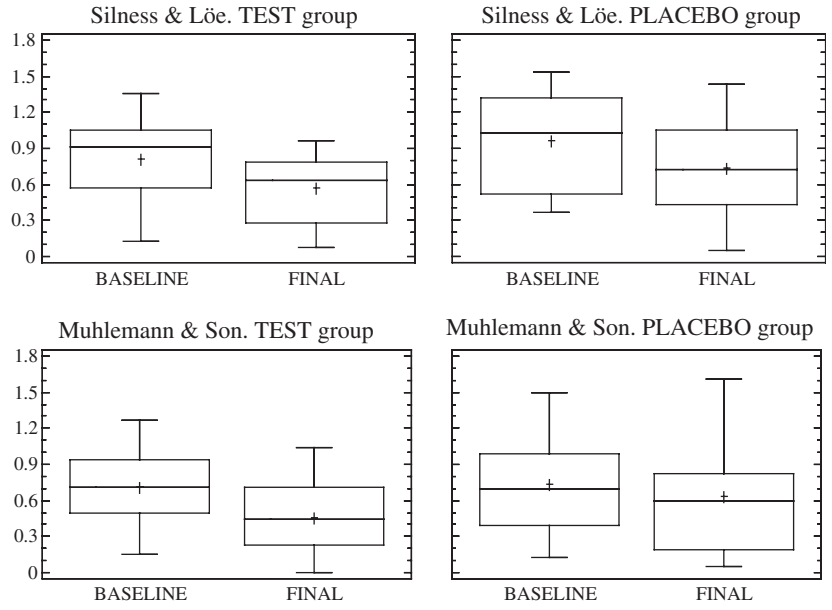

Fig. 2. Box and whisker plots of gingival indices for test and placebo groups, at baseline and final visits.

test group, while in control samples only minor changes occurred (Table 4). Moreover, the percentage of this bacteria in the flora of positive sites significantly ($p = 0.049$) decreased in test samples, while it increased in the placebo group, showing a significant difference between both groups at the final visit ($p = 0.005$).

Prevotella intermedia

The frequency of detection of this pathogen was reduced in both groups. The percentage of this pathogen in the flora was significantly ($p = 0.025$) reduced in the placebo, while in the test group a clear increase was observed. The intergroup comparison also demonstrated statistically significant differences when comparing changes of percentage of flora between visits ($p = 0.02$).

Other pathogens

The results for other bacterial pathogens did not show significant differences (Table 4). Only one or two samples per group at baseline were positive for *A. actinomycetemcomitans* or *B. forsythus*. The presence of these pathogens did not change in control samples; however, in the test samples these pathogens were not detected anymore. Regarding *P. micros*, *F. nucleatum*, and *C. rectus*, the test group tended to increase their percentage of flora, without changing their frequency of detection; while in the placebo samples, a decrease in their frequency of detection was observed, without changing their proportion of flora.

Compliance

Twenty six patients (13 per group) were evaluated for compliance by interview. Eleven out of 13 patients (84.6%) in each group indicated complete compliance, while two in each group revealed a reduced frequency of use.

Staining

Nineteen patients (nine in test group and 10 in control group) were evaluated for staining by means of standardized digital photographs. Two independent judges assessed the pictures, whose results were highly correlated (κ scores). The results from general staining, both with respect to intensity and coverage, showed an increase in the test group, statistically significant for intensity ($p = 0.01$) and coverage ($p = 0.03$). When intergroup comparisons were performed, the test group demonstrated higher increases, but only significant for intensity ($p = 0.02$).

Patient-based variables

Thirty patients (16 test, 14 control) were interviewed to assess oral alterations related with the use of the mouth rinse. One patient (6%) in the test group complained about staining, while none did in the control.

Taste alterations were reported by six patients (37.5%) in the test group, versus none in control. The alterations reported were a decrease in the taste perception (three patients), and a feeling of 'fresher' mouth (one patient), while two patients did not describe the alteration.

Oral alterations were recorded in six patients (37.5%) in the test group, and two patients in the control (14%). Test patients described the oral alteration as a ''burning'' mouth feeling immediately

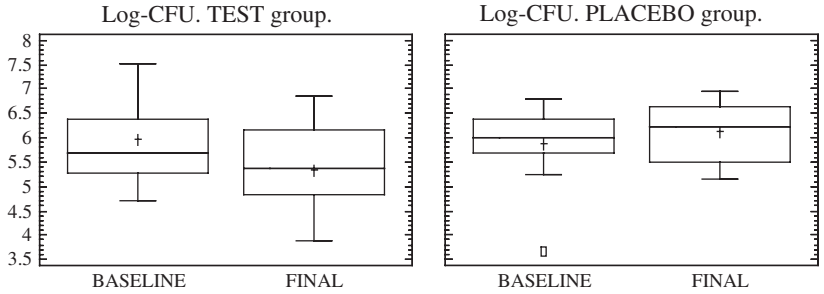

Fig. 3. Box and whisker plots of log-CFU for test and placebo groups, at baseline and final visits.

Table 4. Frequency of detection (percentage) and mean proportions of total flora, in positive sites, for selected pathogens, at each visit and for each treatment group

	Test group		Placebo group	
	baseline	final	baseline	final
Porphyromonas gingivalis				
frequency	9/14 (64%)	3/16 (19%)	10/13 (77%)	10/14 (71%)
% of flora	21	17	19	30
Prevotella intermedia				
frequency	9/14 (64%)	9/16 (56%)	11/13 (85%)	9/14 (64%)
% of flora	3.5	9.5	8	2
Peptostreptococcus micros				
frequency	5/14 (36%)	8/16 (50%)	9/13 (69%)	6/14 (43%)
% of flora	6.4	15.2	1.7	2.1
Fusobacterium nucleatum				
frequency	11/14 (79%)	13/16 (81%)	12/13 (92%)	11/14 (79%)
% of flora	5.1	11.2	8.4	7.9
Campylobacter rectus				
frequency	4/14 (29%)	5/16 (31%)	5/13 (38%)	3/14 (21%)
% of flora	3.8	17.2	0.9	2.7

after use (three patients), or a ''dry-mouth'' sensation (three patients).

Other observations were pointed out by two test patients (13%) and two control patients (14%). Test patients reported an overall oral ''improvement'' (one patient) or a feeling of ''cleaner mouth'' (one patient), while control patients described a ''cleaner mouth'' (one patient), or more tooth hypersensitivity (one patient).

Discussion

The results of the present short-term randomized clinical trial, showed that a low CHX concentration mouth rinse can decrease plaque levels in patients in supportive periodontal care. The reduction in plaque was associated to a reduction in total anaerobic subgingival microflora. Concomitantly, a decrease in the frequency of detection and proportions of flora of *P. gingivalis* was observed.

Reductions in the level of plaque and gingivitis were statistically significant in the intragroup assessment in the test group, while changes in plaque between visits were statistically significant different between the test and placebo groups. However, no intergroup difference was detected regarding gingival indices. This was not related to an absence of antigingivitis activity, since, as mentioned before, a statistically significant reduction was observed in the intragroup evaluation in test patients. Rather, a concomitant decrease in the control group, although not significant, could be responsible of the absence of intergroup differences. Different reasons could explain this finding: it has been suggested that short-term studies on oral hygiene products can minimize differences among products, due to the initial Hawthorne effect (Mauriello et al. 1987, Graves et al. 1989), and this effect could have improved the results in both groups, masking possible existing differences; moreover, patients in supportive care knew the importance of oral hygiene in their periodontal health, and their motivation and compliance may have improved between the evaluation visits, in spite of the absence of any treatment; and lastly, the influence of the reduction of *P. intermedia* levels in the control group is unclear, although its presence has been related with gingival inflammation (Haffajee & Socransky 1994).

The test mouth rinse demonstrated a significant effect in the subgingival bacterial load (both intra- and intergroup), together with a reduction in the levels of *P. gingivalis* (only at an intragroup level). This outcome agrees with recent studies that have shown an important effect of different oral hygiene products on the subgingival microflora, both as toothpastes with triclosan/copolymer (Rosling et al. 1997), and as mouth rinses with CHX/CPC/zinc lactate (Roldán et al. 2003). Whether this subgingival activity was related to changes in the subgingival niche, the supragingival plaque, or a true subgingival antimicrobial effect, is something that remains unclear.

The test product has been designed for long-term use in patients in supportive periodontal care. Firstly, CHX concentration has been reduced to 0.05%, which might decrease the known adverse effects of this agent as compared with 0.12–0.2% concentrations, although this has not been evaluated in the present study. In the present study, the test product produced more staining than the placebo formulation, and was already significant after 2 weeks of use, in terms of intensity. However, this fact was only identified at an examiner level, since only one patient in the test group had complaints of staining at the interview. Other oral alterations, although more frequent in the test group, were mainly identified as positive by the patients, such as a feeling of a fresher or cleaner mouth after rinsing. Secondly, no alcohol was included in the composition. The inclusion of alcohol in mouth rinses is still a controversial subject. The presence of alcohol in oral hygiene products aimed for long-term use has been associated to different drawbacks (oral cancer, mucositis, etc.) (Winn et al. 1991, Shapiro et al. 1996), in spite of clear advantages, such as a higher antimicrobial activity (Herrera et al. 2003), and a decrease in the risk of bacterial contamination of the product itself (Vigeant et al. 1998).

To compensate for the low CHX concentration and the absence of alcohol, CPC was added to the formulation. It has been shown that the addition of CPC to the formulation could compensate the

lack of alcohol in 0.12% CHX formulations and even improve its activity (Herrera et al. 2003, Quirynen et al. 2001). Regarding the low CHX concentration, plaque inhibition by CHX is dose-dependent, and low-concentration rinses are less effective, but still can be considered as adjuncts to oral hygiene (Jenkins et al. 1994). Recently, a mouth rinse with 0.05% CHX, formulated to treat oral halitosis (also with 0.05% CPC, 0.12% zinc chloride, and no alcohol; Halita®, Dentaid, Barcelona, Spain), has also demonstrated both efficacy in reducing halitosis (Winkel et al. 2003), as well as modifying the microbiological oral environment, including the subgingival microflora (Roldán et al. 2003).

The results of the present study demonstrate that the test product is effective in reducing plaque levels and the anaerobic subgingival microflora. However, these results are only short term, and this plaque-inhibitory activity needs to be confirmed in a 6-month, home-use, clinical trial (Wu & Savitt 2002). Moreover, in a longer trial, an antiplaque effect could be detected, if the Hawthorne effect had affected the gingival index results of the present study.

Conclusion

The test mouth rinse demonstrated plaque-inhibitory activity in a 2-week clinical trial, and this was associated with a reduction in the total subgingival anaerobic microflora.

Acknowledgments

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