J Clin Periodontol 2005; 32: 390–400 doi: 10.1111/j.1600-051X.2005.00685.x

Journal of Clinical Periodontology

A 0.05% cetyl pyridinium chloride/ 0.05% chlorhexidine mouth rinse during maintenance phase after initial periodontal therapy

Marc Quirynen[1,2], Catherine Soers[1], Mandy Desnyder[1], Christel Dekeyser[1], Martine Pauwels[2] and Daniel van Steenberghe[1,*]

[1]Department of Periodontology and [2]Research Group for Microbial Adhesion, School of Dentistry, Oral Pathology and Maxillo-Facial Surgery, Faculty of Medicine, Catholic University of Leuven, Leuven; [3]Laboratory for Statistics and Experimental Design, Faculty of Agriculture, Catholic University of Leuven, Heverlee, Belgium

Quirynen M, Soers C, Desnyder M, Dekeyser C, Pauwels M, van Steenberghe D. A 0.05% cetyl pyridinium chloride/0.05% chlorhexidine mouth rinse during maintenance phase after initial periodontal therapy. J Clin Periodontol 2005; 32: 390–400. doi: 10.1111/j.1600-051X.2005.00685.x. © Blackwell Munksgaard, 2005.

Abstract

Background: Chlorhexidine (CHX) mouth rinse/spray can still be considered the gold standard in the chemical prevention of plaque formation and development of gingivitis. The product unfortunately has some side effects, such as extrinsic tooth staining, poor taste, taste disturbance, sensitivity changes in tongue, pain and irritation because of the alcohol content. These side effects led to the search of new formulations.

Methods: In this double-blind, randomized, long-term, parallel study, 48 moderate periodontitis patients rinsed for 6 months (starting immediately after a ''one-stage, full-mouth'' disinfection) with one of the following products: CHX 0.2%+alcohol (Corsodyl®), CHX 0.05% + cetyl pyridinium chloride (CPC) 0.05% and no alcohol (Perio-Aid Maintenance®, a new formulation), or the placebo of the latter. After 1, 3 and 6 months a series of clinical and microbiological parameters were recorded for the supra- and subgingival area as well as for saliva.

Results: Although there was a significant treatment impact (mechanical debridement) in all groups, both CHX solutions further decreased both plaque and gingivitis indices ($p<0.001$ and $p<0.05$, respectively), when compared with placebo. This was also reflected by additional reductions in the number of CFU/ml of aerobic and especially anaerobic species and by a suppression of *Streptococcus mutans* (*versus* an overgrowth for the placebo), in all niches. Differences between both CHX solutions were never encountered. The subjective ratings were slightly in favour of the new CHX–CPC formulation when compared with the other CHX–alcohol formulation, especially for taste of the product ($p<0.05$), but less impressive for the staining of teeth and tongue.

Conclusions: The results of this study demonstrated the potential of a new CHX 0.05%+CPC 0.05% non-alcoholic formulation as an effective antiplaque agent for long-term use with reduced subjective side effects.

Key words: chemical plaque agents; chlorhexidine; clinical studies; dental plaque; gingivitis; microorganisms; mouth rinses; periodontitis; plaque; prevention; supragingival plaque control

Accepted for publication 12 August 2004

Optimal plaque control still is the cornerstone of a successful treatment of periodontitis and/or in maintaining periodontal health. Supragingival plaque control may be considered as a ''sanitary'' procedure that lowers the levels of potentially pathogenic species in the oral cavity. As such, this reduction in the reservoir of potentially pathogenic organisms is of major importance in lowering the risk of disease recurrence in susceptible individuals (Socransky et al. 2002, Socransky & Haffajee 2002). Moreover, several key papers clearly illustrated that subgingival debridement and/or periodontal surgery are only successful when combined with optimal plaque control and/or repeated professional cleaning (Lindhe & Nyman 1975, Nyman et al. 1975, Rosling et al. 1976, Axelsson & Lindhe

*Holder of the Brånemark chair in osseointegration.

1981a, b, Lindhe et al. 1982, 1984, Axelsson et al. 1991, Westfelt et al. 1998, Ximenez-Fyvie et al. 2000, Checchi et al. 2002).

Supragingival plaque control has also an added benefit because it also affects both the numbers and the composition of the subgingival microbiota. This may be because of a direct effect of the supragingival colonizers on subgingival organisms and/or an effect on the adjacent periodontal tissues (healing of periodontium has indirect effect on subgingival flora). Indeed, the removal of the supragingival plaque and the resulting improvements in the gingival margin will reduce the essential growth requirements for the subgingival flora so that bacterial numbers will decrease spontaneously (Socransky & Haffajee 2002). This beneficial aspect has been illustrated in several clinical trials examining the impact of repeated supragingival professional cleaning on the subgingival flora (Dahlen et al. 1992, al Yahfoufi et al. 1995, Hellstrom et al. 1996).

Antiplaque agents can be recommended in situations in which oral hygiene is difficult, compromised or impossible (Addy & Moran 1997a). Chlorhexidine (CHX) mouthrinse, spray and/or gel can be considered as the gold standard for oral antiseptics (for review see Addy 1986, Addy et al. 1994, Bollen & Quirynen 1996, Jones 1997, Addy & Moran 1997b). The antibacterial activity of CHX was found to be dosage dependent, with 0.1% being a threshold level above which no further benefits can be expected (Bonesvoll et al. 1974, Jenkins et al. 1994a, b, Smith et al. 1995, Ernst et al. 1998). Unfortunately, CHX, as most active antiseptics, has some disadvantages (Flotra et al. 1971, Loe et al. 1976). It infrequently irritates the oral mucosa in a non-dosage-dependent matter. Discolouration of the pellicle, especially in the inter-proximal areas, and tongue are often encountered. The former is caused by a precipitation reaction between tooth-bound CHX and chromogens from food or beverages, or can be smoking related (Addy et al. 1991a, b). Nordbo et al. (1982) linked this staining to the denaturing capacity of CHX by which the released iron can participate in the staining. CHX also has a reversible effect on the taste (intensity and quality) for NaCl and quinine–HCL, and to a lesser extent on the taste quality of sucrose and citric acid (Helms et al. 1995). Most CHX rinses, contain ethanol which can cause a dose-related pain (Bolanowski et al. 1995). Some of them may contain 25% alcohol or more and could eventually increase the risk for oral cancer, especially in regular users of mouth rinses (Wynder et al. 1983, Smigel 1991, Winn et al. 1991). Although the relationship between alcohol-containing mouth rinses and the prevalence for oral cancer remains a matter of debate, because of a number of confounding variables (Elmore & Horwitz 1995, Shapiro et al. 1996), the above-mentioned observations are further impetus for the development of non-alcohol-containing mouth rinses. Moreover, for some patient populations (mucositis, head and neck irradiation, immuno-compromised, alcoholics) alcoholic solutions are contraindicated (Eldridge et al. 1998). Alcohol – especially ethanol – is a common chemical agent to dissolve substances in mouth rinse solutions. In some mouth rinses containing CHX it is added to prevent product contamination, for example by *Pseudomonas* spp. By itself, ethanol possesses only a slight antibacterial efficacy against oral bacteria in vitro and in vivo and will only be efficient when its concentration is $>50\%$ (Gjermo et al. 1970, Sissons et al. 1996).

In this perspective, the present study aimed to examine the antibacterial capacity and side effects of a new CHX solution with a lower concentration of CHX (0.05%) in combination with 0.05% cetyl pyridinium chloride (CPC) and without ethanol, over a 6-month period. Several papers already highlighted that an alcohol-free solution containing 0.12% CHX+0.05% CPC forms a good alternative to the existing alcohol-containing CHX solutions (Quirynen et al. 2001, Herrera et al. 2003).

Material and Methods

Subjects

Sixty-one Caucasians volunteered for this study, but only 48 (mean age = 48.8 years, 27 females) completed the 6 months clinical trial. They all suffered from moderate-to-severe periodontitis [at least two multi-rooted and two single-rooted teeth per quadrant with probing depths $\geqslant 6$ mm, and radiographic evidence of bone loss ($\geqslant 1/3$ the root length)]. All subjects were in good general health and none of them had used any antimicrobials during 4 months prior to the study. After an explanation of the therapy, all participants signed an informed consent form. The protocol had been favourably reviewed by the Clinical Trials Committee of the University Hospital. Patients ($n = 13$) dropped out from the study, mostly because of the intake of antibiotics [(often for a severe cold ($n = 8$) or for another pathology ($n = 2$)], or because of medical complication ($n = 3$) not linked to the oral pathology. The dropout group did not differ significantly from the group completing the study, except for a slightly higher proportion of smokers.

Experimental design

A double-blind, randomized, 6-month clinical trial with parallel grouping was set up to follow the long-term effect (e.g. clinical efficacy, adverse effects, microbial shifts, Table 1) of three mouth rinses as maintenance product. The patients were selected by a clinician who evaluated the inclusion and exclusion criteria (the latter including recent intake of antibiotics, medical history rendering antibiotic prophylaxis during periodontal therapy mandatory, anticoagulance, chemotherapy, irradiation in head and neck area, etc.). This clinician was masked for the allocation of the rinses.

At baseline all patients received a ''one-stage, full-mouth disinfection'' (Mongardini et al. 1999, Quirynen et al. 1999a) including a scaling and root planing of all pockets within 24 h (local anaesthesia) with standard periodontal curettes, immediately followed by an additional disinfection of all intra-oral niches for periopathogens via a subgingival irrigation of all the pockets (three times within 10 min.) with a 1% CHX gel (Corsodyl gel®, GlaxoSmithKline, Genval, Belgium), tongue brushing (by the patient) for 60 s with the same gel, and rinsing twice with a 0.2% CHX solution (Corsodyl gel®, GlaxoSmithKline) for 1 min.

All subjects were instructed to rinse twice daily for 1 min. with one of the three test rinses for a period of 6 months. The rinses were coded to prevent any bias and the codes were not broken before the end of the study. The patients were randomly allocated by a second clinician (uninformed about the protocol) to one of the following rinses

Table 1. Flow chart of the study including pre- and post-study activities

Investigational events	Prior to study	Baseline	Week	Month		
			1	1	3	6
Medical history	x					
Dental status	x					
Intra-oral long-cone RX	x					
Periodontal status	x					
Periodontal status 1 quadrant		x			x	x
One-stage full-mouth disinfection		x				
Allocation to rinse		x				
Oral hygiene control		x	x	x	x	x
Oral hygiene instruction		x	x	x	x	x
Polishing				x	x	x
Plaque samples						
Saliva		x			x	x
Supragingival plaque		x			x	x
Subgingival		xx			xx	xx
Plaque indices ($n = 2$)		x		x	x	x
Gingivitis indices ($n = 2$)		x		x	x	x
Staining indices		x		x	x	x
Soft-tissue conditions		x		x	x	x
Questionnaires			x	x	x	x
Taste perception		x			x	x
Adverse events			x	x	x	x

RX, radiographs.

(by the order of intake and correcting at the end for the dropouts):

- a positive control rinse ($n = 16$): 10 ml of CHX 0.2% in alcohol (Corsodyl, GlaxoSmithKline; abbreviation CHX–Al);
- a test solution ($n = 16$):15 ml of 0.05% CHX and 0.05% CPC as active ingredients but without alcohol (Perio-Aid Maintenance®, Dentaid, Barcelona, Spain, abbreviation CHX–CPC)
- or a placebo ($n = 16$) of the test solution (15 ml) containing all ingredients except CHX and CPC (abbreviation Pla).

The patients were instructed to leave at least 1/2 h between toothbrushing and rinsing, in order to prevent an inactivation of the CHX by detergents in the toothpaste (Barkvoll et al. 1989). During the entire period of 6 months, no further subgingival instrumentation was allowed.

Oral hygiene

All patients received standard oral hygiene instructions immediately after the first session of scaling and root planing including inter-dental plaque control (inter-dental brushes, Interprox®, Dentaid) and tongue cleaning (tongue scraper, Halita®, Dentaid) twice a day. All patients were also provided with the same toothbrushes (Vitis® Suave, Dentaid) and the same toothpaste (Fluor Aid 250®, Dentaid). Oral hygiene control and re-instruction were given at several occasions (week 1, and months 1, 3 and 6, respectively). Moreover, at the 1-, 3- and 6-month recall visits, all teeth were polished to remove eventual staining.

Periodontal parameters (Table 1)

The following parameters (in sequential order) were recorded prior to subgingival debridement (baseline), and at the end of months 1, 3 and 6):

- tooth staining using the Quigley & Hein (Turesky et al. 1970) plaque index for the Ramfjord teeth (16, 21, 24, 36, 41, 44; Rams et al. 1993);
- plaque surface extension (after disclosure with a 4% aqueous erythrosin solution) assessed using the Quigley & Hein (Turesky et al. 1970) and the modified Navy plaque indices (Hancock & Wirthlin 1977) for same teeth;
- degree of gingival inflammation along Ramfjord teeth using the sulcus bleeding index (Muhlemann & Son 1971), with six sites per tooth (mesially, centrally and distally both buccally as well as orally), the scores ranging from 0 to 5; and along the contra-lateral teeth (14, 11, 26, 34, 31, 46) using the papillary bleeding index (Saxer & Muhlemann 1975), with four sites per tooth (approximal area) the scores ranging from 0 to 4.

These indices are considered very reliable (Marks et al. 1993, Newbrun 1996), and the validity of the Ramfjord teeth to replace a full-mouth recording has previously been confirmed earlier (Silness & Roynstrand 1988).

The following clinical parameters (in sequential order) were recorded immediately after debridement and at the end of months 3 and 6, in the right maxillary quadrant (reference quadrant):

- the probing depth to the nearest 0.5 mm (buccally and orally of each root, and at each approximal site, both buccally and orally) by means of a Merrit B® probe (Hu-Friedy, Chicago, IL, USA);
- the bleeding tendency evaluated 20 s after probing the depth of the pocket; the scores are 0 (absent) or 1 (present).

Microbial samples

The following four samples were collected at baseline, and after 3 and 6 months (Table 1), respectively:

- 0.2 ml saliva [representative for the microbial load in the oral cavity, (Umeda et al. 1998) collected in 1.8 ml (Syed & Loesche 1972)];
- all supragingival plaque from the buccal and palatal surfaces of the two teeth (e.g. 16, 24) removed with sterile curettes and pooled in one cup with 2 ml reduced transpost fluid (RTF);
- the subgingival flora of single- and multi-rooted teeth in the maxillary right quadrant (pooled samples into a cup containing 2 ml RTF-from the three deepest approximal sites/tooth type at baseline); the selected sites were cleaned supragingivally (sterile curettes) prior to sampling, isolated from saliva (cotton rolls) and gently dried to prevent contamination; per site, four sterile medium paper points (Roeko®, Langenau, Germany) were inserted (two buccal and two lingual) and kept in place for at least 10 s.

Periopathogens

Aliquots of 0.1 ml of the first three dilutions were plated manually for the

detection of *Actinobacillus actinomycetemcomitans* on a highly selective medium (Alsina et al. 2001). The dilutions 10^{-1}–10^{-5} were plated in duplicate onto non-selective blood agar plates. After 7 days of anaerobic incubation and aerobic incubation at 37°C, the total number of CFU/ml were counted. For each pigmented (black, green, brown) colony ''type'' (further referred to as b.p.b. = black pigmented bacteria) on the representative anaerobic plate, every third colony was subcultured and identified (for details see Quirynen et al. 1999a). The dilutions 10^{-1}–10^{-5} were also plated on two additional selective media: the Hammond medium for the detection of *Campylobacter rectus* and the CVE medium for the detection of *Fusobacterium nucleatum* (Walker et al. 1979, Hammond 1988).

Cariogenic species

For the detection and identification of cariogenic species, serial 10-fold dilutions (up to 10^{-5}) were plated on selective media:

- TYCSB medium (trypticase, yeast, cystine agar, supplemented with 40 w/v Sucrose and 0.2 U/ml Bacitracin), for the isolation of *Streptococcus mutans* and *Streptococcus sobrinus*;
- Rogosa medium (Bacto Rogosa SL agar, Difco®, Led Techno, Eksel, Belgium) for lactobacilli (Rogosa et al. 1951, Schupbach et al. 1995).

Questionnaire (visual analogue scale)

At each follow-up visit (week 1, and months 1, 3 and 6) the subjects were asked to complete, anonymously, a questionnaire concerning the taste of the product, loss of taste, special sensations on the tongue or mucosae, and the staining of their teeth and tongue. A last question estimated the compliance of the patient by asking whether the patient followed the rinse instructions strictly. All questions were answered by making a cross mark on a 100 mm line with the most extreme answers at both ends.

Taste sensation

At baseline and during the 3 and 6 months follow-up, all subjects were submitted to recognition tests for salt, sweet, sour and bitter. For all tastes (sodium chloride, saccharose, citric acid and quinine), different concentrations (with 1/3 concentration reductions) were prepared. Starting with the lowest concentration of a randomly chosen taste, one drop was put on the tongue, using a dropping bottle. The concentration was increased in successive trials until the given taste was correctly identified (recognition threshold identification, Helms et al. 1995).

Statistical analysis

The hypotheses of this study were that the two CHX rinses acted equally efficient when compared with each other, but significantly better than the placebo. For all analyses the patient was considered the statistical unit by calculating per patient a mean value for each variable. Moreover, transformations (e.g. a logarithmic, a square power or a square root) were carried out in order to satisfy the assumptions of normally distributed error terms.

A linear mixed model was fitted taking into account the patient as a random factor, and the mouth rinse as fixed factor (together with the experimental period if the latter was of significant importance according to the Akaike's information criteria). When data were obtained over several follow-up visits, the factor month was also included. Under the latter condition, the patient was also considered as a subject for repeated measurements. Differences between mouth rinses and/or with baseline values were looked for via a set of pair-wise comparisons, corrected for simultaneous hypothesis testing using the Tukey–Kramer method for multiple comparisons. Before each analysis, the residuals were tested for normality by a normal QQ-plot. In case of a deviation from normality, data were transformed by a log or power transformation.

Results

Plaque indices

Both plaque indices [modified Quigley & Hein (Turesky et al. 1970) and modified Navy plaque index (Hancock & Wirthlin 1977)] reduced significantly for all three groups (Fig. 1) after mechanical debridement and oral hygiene instruction (treatment effect, $p<0.001$). The CHX–Al and CHX–CPC rinse, although never significantly different from each other (p always >0.5), showed a significant additional reduction in plaque scores, when compared with placebo ($p<0.002$), and this for all follow-up visits (p values ranging from 0.05 to 0.0005 depending on follow-up visit, 0.005 especially for 3- and 6-month observations). For all follow-up visits, CHX–Al and CHX–CPC rinsing resulted in values below 50% of that of the placebo rinse. These additional improvements were most impressive for the oral surfaces when the Navy plaque index was considered.

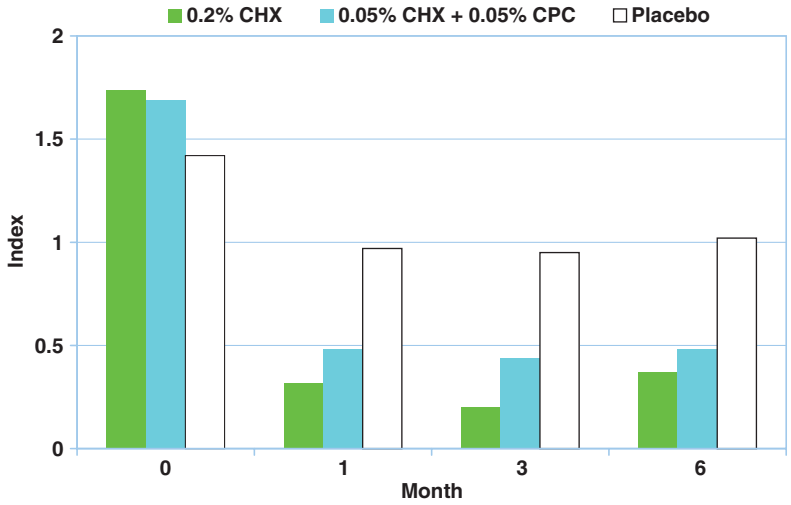

Fig. 1. Mean Quigley & Hein plaque index over a 6-month follow-up period of daily plaque control in combination with twice daily use of a mouth rinse [0.2% chlorhexidine (CHX) + alcohol *versus* 0.05% CHX+0.05% cetyl pyridinium chloride (CPC) without alcohol *versus* placebo] sorted per rinse (with 16 subjects per product) and per follow-up visit (before mechanical debridement, and after 1, 3 and 6 months).

Gingival indices

Consequently, also the degree of gingival inflammation [scored via the Sulcus Bleeding index (Muhlemann & Son 1971) or the papillary bleeding index (Saxer & Muhlemann 1975)] reduced significantly over time (Figs. 2a and b) for all rinses (treatment effect, $p<0.001$). Besides this significant treatment effect, both the CHX–Al and the CHX–CPC rinses resulted in a small but statistically significant additional improvement in gingival health ($p<0.05$ for sulcus bleeding index, $p=0.10$ for papillary bleeding index), especially at the 6-month follow-up were the placebo group shows some relapse. At the 6-month observation, the gingivitis indices for both CHX rinses were about half the value of that for the placebo rinse.

Tooth staining

The degree of tooth staining significantly increased ($p<0.001$) for both CHX rinses, and this in contrast to the placebo solution (Fig. 3). Between the CHX rinses the differences were negligible ($p>0.05$), but always lower for the CHX–CPC rinse.

Probing depth

The probing depths reduced for all three test groups, with a significant treatment effect ($p<0.001$). Initially, deep pockets ($\geqslant 7$ mm) improved from an overall mean depth of 7.2 and 7.4 mm for single and multi-rooted teeth, respectively, to 4.9 and 5.3 mm. For the medium pockets (4–6 mm) the corresponding changes were from 4.7 and 4.8, to 3.5 and 3.8 mm, respectively. The impact of the two CHX-containing rinses when compared with the placebo solution was, however, negligible.

Also the bleeding on probing tendency significantly decreased after therapy ($p<0.0001$) from around 70% to 30%. Both CHX rinses always scored superior reductions when compared with the placebo, but the differences were borderline significant ($p=0.06$ for product effect).

Microbiological data

In contrast to the placebo group where the total number of CFU/ml in samples from the supragingival plaque showed only negligible changes over time (reductions $\leqslant 0.5$ log values, especially for the aerobic flora $p>0.20$), significant reductions with time ($p<0.001$) were detected in both CHX groups and for both aerobic as well as for anaerobic species (reductions around 1.0 log values, $p<0.001$, Fig. 4). Differences between the CHX–Al and CHX–CPC rinses were never detected (p always >0.90, for each follow-up visit).

The changes in the microbial load within the saliva are also shown in Fig. 4. For the placebo solution, the number of CFU/ml aerobic or anaerobic species did not show any change (data within 0.1 log value). The two CHX solutions showed some small, but statistically significant ($p<0.05$) reductions ($\geqslant 0.4$ log value) when compared with baseline, for both aerobic and anaerobic species. The differences between both CHX rinses were again negligible ($p>0.90$).

The changes in subgingival microbial load around single- and multi-rooted teeth, respectively, are depicted in Fig. 5. The number of aerobic as well as anaerobic species around single-rooted teeth reduced significantly (up to 1.0 log value) for both CHX groups ($p<0.005$ at month 3, $p<0.05$ at month 6 for aerobic and $p<0.005$ for anaerobic species, respectively), but remained nearly unchanged for the placebo group (small treatment effect with a 0.3 log reduction). Between CHX–Al and CHX–CPC rinses, significant differences were never detected (p always >0.50, for each follow-up visit) and the

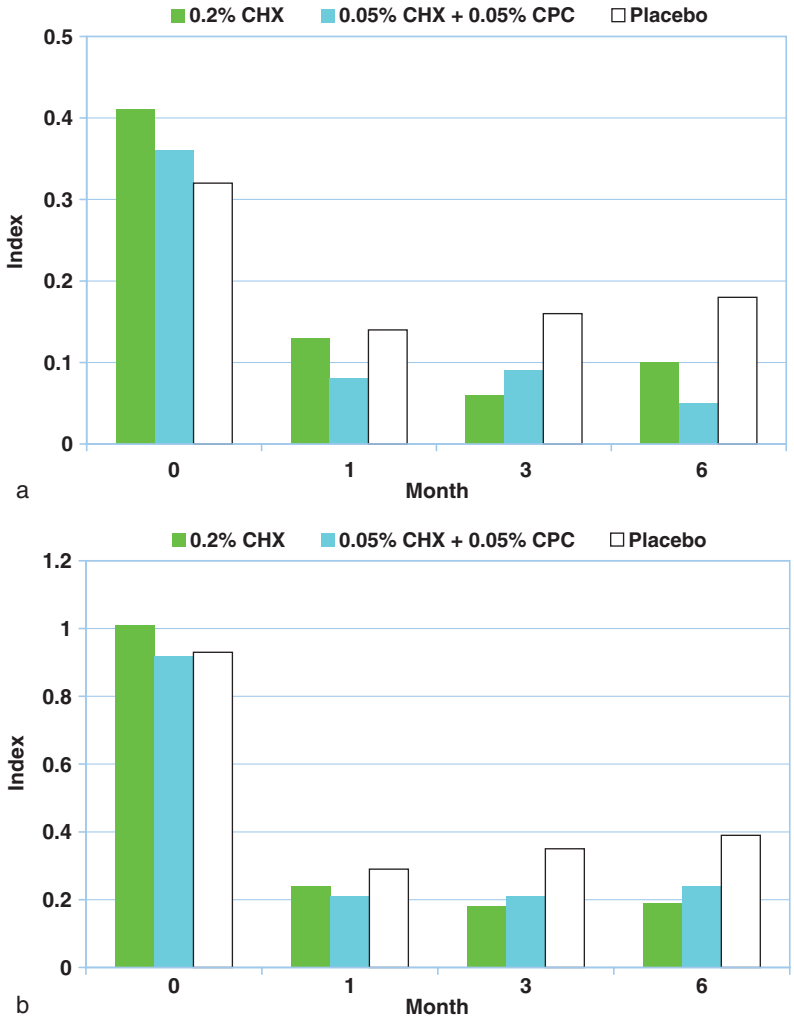

Fig. 2. Mean gingivitis indices [(a) sulcus bleeding index (Muhlemann & Son 1971); (b) papillary bleeding index (Saxer & Muhlemann 1975)] over a 6-month follow-up period of daily plaque control in combination with twice daily use of a mouth rinse [0.2% chlorhexidine (CHX) + alcohol *versus* 0.05% CHX + 0.05% cetyl pyridinium chloride (CPC) without alcohol *versus* placebo] sorted per rinse (with 16 subjects per product) and per follow-up visit (before mechanical debridement, and after 1, 3 and 6 months).

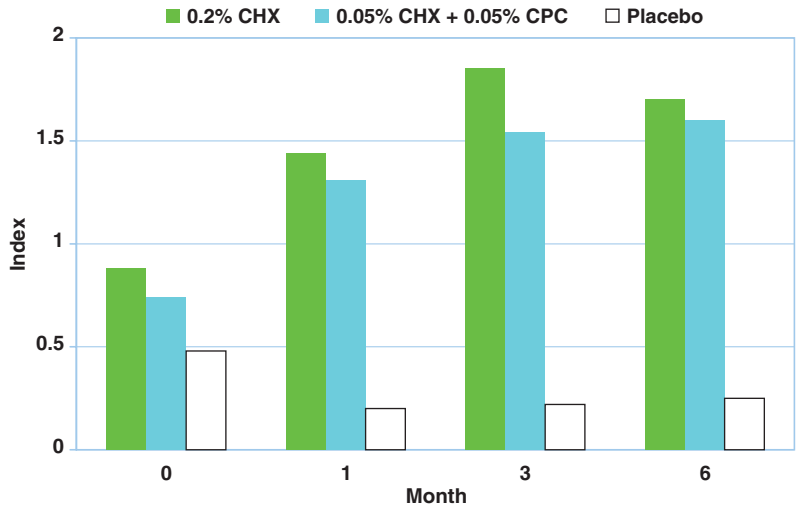

Fig. 3. Mean degree of tooth staining (scored via the Quigley & Hein plaque index) over a 6-month follow-up period of daily plaque control in combination with twice daily use of a mouth rinse [0.2% chlorhexidine (CHX) +alcohol *versus* 0.05% CHX+0.05% cetyl pyridinium chloride (CPC) without alcohol *versus* placebo] sorted per rinse (with 16 subjects per product) and per follow-up visit (before mechanical debridement and after 1, 3 and 6 months).

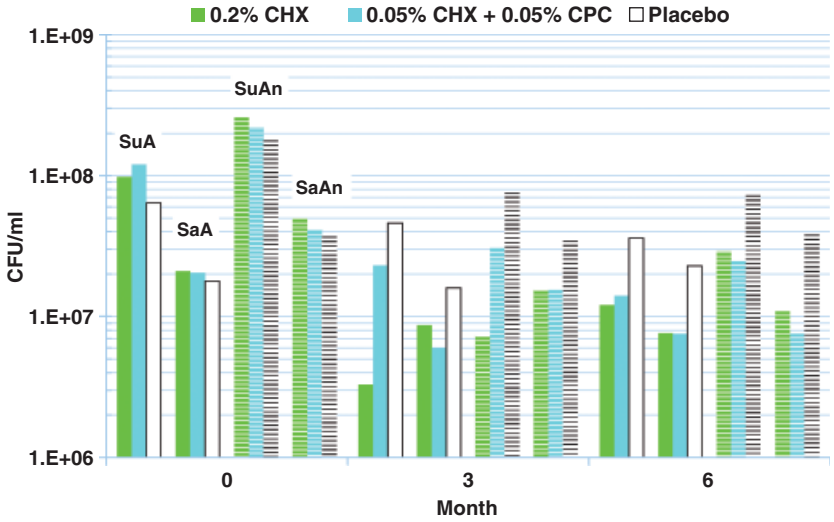

Fig. 4. Mean changes in total number of CFU/ml (aerobic ''A'' and anaerobic ''An'' culturing) in samples from the supragingival plaque (Su) and from the saliva (Sa) over a 6-month follow-up period of daily plaque control in combination with twice daily use of a mouth rinse [0.2% chlorhexidine (CHX) +alcohol *versus* 0.05% CHX+0.05% cetyl pyridinium chloride (CPC) without alcohol *versus* placebo] sorted per rinse (with 16 subjects per product) and per follow-up visit (before mechanical debridement and after 3 and 6 months).

changes obtained at month 3 could be maintained up to month 6. For the multi-rooted teeth the changes were comparable with similar intra- as well as inter-product variations.

Finally, the detection frequency of specific cariogenic and periopathogenic species are summarized in Table 2. It is obvious that the detection frequency for *S. mutans* clearly decreased with both CHX-containing mouth rinses, but increased with the placebo solution. For the latter rinse, the number of CFU/ml for *S. mutans* slightly increased with time. The detection frequency for *Lactobacilli* species decreased in the two CHX groups, and remained stable in the placebo group. For the periopathogens *Porphyromonas gingivalis* and *Prevotella intermedia* significant reductions were recorded after therapy, but again less impressive for the placebo solution. *A. actinomycetemcomitans* was only sporadically detected (in 7/48 patients) and only at baseline, but never at the 3- or 6-month follow-up visits.

Subjective evaluations

The outcome of the anonymous questionnaires, with VAS ratings, is depicted in Fig. 6. The CHX–Al rinse scored significantly worse ($p = 0.004$) for its taste when compared with the CHX–CPC combination, which by itself was not considered different from placebo ($p = 0.21$). Both CHX solutions scored significantly worse, when compared with the placebo, concerning loss of taste perception ($p < 0.0001$) and degree of tooth staining ($p < 0.02$ for CHX–Al, 0.08 for CHX–CPC). For the staining of the tongue only the CHX–Al rinse scored significantly worse than the placebo ($p = 0.02$).

The compliance of the patients with the rinsing instructions was similar for the three test products ($p = 0.16$), although difference in time could be detected (reduced compliance over time, $p < 0.001$). In general, the degree of compliance dropped from 95% after 1 week (data not shown) to around 75% at the end of the study. Inter-product differences could thus not be detected ($p > 0.50$).

Changes in taste recognition

The changes in taste recognition are summarized in Fig. 7. Obvious changes in time or between products were not detected.

Discussion

This paper presents the data of a long-term, double-blind study where CHX solutions were used as an adjunct to mechanical debridement in a group of patients with moderate-to-severe periodontitis. In general, the two CHX-containing mouth rinses improved the supragingival plaque control and had an additional beneficial effect on both the degree of gingival inflammation and the microbial load within the oral cavity. Both CHX solutions resulted in an adjunctive reduction (on top of the treatment effect because of debridement) in bacterial load of 0.5–1.0 log value for all examined niches (supragingivally, subgingivally, and to a lower extent in the saliva). Especially the

beneficial effect of both CHX solutions on the subgingival flora is somewhat surprising as it is known that mouth rinses normally do not penetrate subgingivally. Our observations indicate a retardation of the subgingival re-colonization/maturation by the impact of the CHX solutions either on the supragingival plaque and/or on the periodontal tissues. Both rinses indeed showed, besides reduced plaque scores, an additional reduction in the bleeding on probing tendency. Although not significantly different from placebo (due to the important treatment effect by itself), both rinses also resulted in a more stable probing depth reduction (the placebo group showed a tendency for relapse over time).

Several recent papers examined the role of alcohol, especially ethanol, in the antibacterial activity of CHX-containing mouth rinses. In general, it was observed that the removal of the alcohol from the rinse had no, or only a minor impact on the antiplaque activity of the other active ingredients (Eldridge et al. 1998, Quirynen et al. 2001, Leyes Borrajo et al. 2002, Herrera et al. 2003).

The data of this study, as far as the knowledge of these researchers goes, illustrate for the first time, in vivo, that the replacement of alcohol in a CHX formulation by CPC did not change the antibacterial activity, even though the CHX concentration (0.05%) was reduced below the 0.10% threshold level often suggested as the optimal antibacterial activity (Jenkins et al. 1994b, Smith et al. 1995, Ernst et al. 1998). Recently, two other papers already reported on the clinical and microbiological benefits of a 0.12% CHX+0.05% CPC formulation without alcohol, and indicated that this new formulation was indeed very promising (Quirynen et al. 2001, Herrera et al. 2003). An in vitro antimicrobial activity test (1 min. contact time) showed that the new formulation was even more efficient than a 0.12% CHX solution with alcohol in killing specific periopathogens and cariogenic species (Herrera et al. 2003). An in vivo test indicated that the new formulation was equally efficient in reducing the bacterial load in the saliva over a period of 5 h (Herrera et al. 2003), when compared with a CHX 0.12%+alcohol solution, but significantly superior to a CHX 0.12% without-alcohol solution, or a combination of CHX and sodium fluoride (Herrera et al. 2003). In our previous project on the effect of different CHX formulations on the de novo plaque

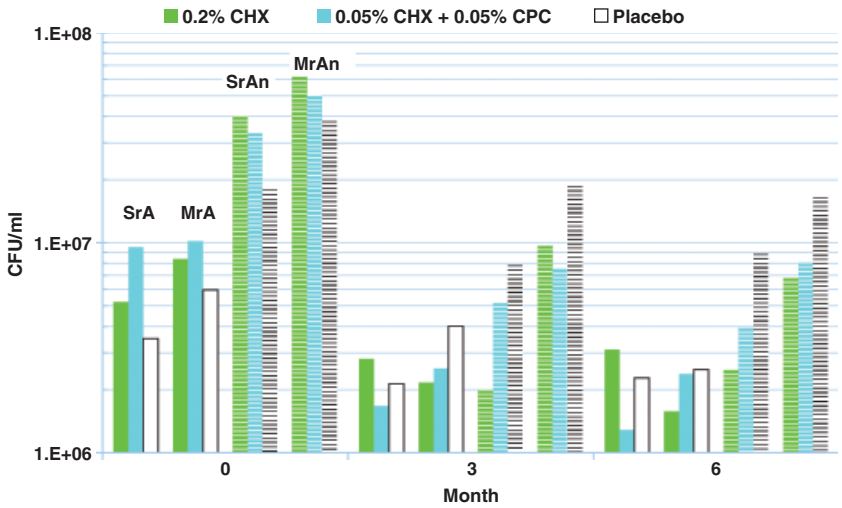

Fig. 5. Mean changes in total number of CFU/ml (aerobic ''A'' and anaerobic ''An'' culturing) in samples from the subgingival flora [pooled samples from single (Sr) and multi-rooted (Mr) teeth, respectively], over a 6-month follow-up period of daily plaque control in combination with twice daily use of a mouth rinse [0.2% chlorhexidine (CHX) +alcohol *versus* 0.05% CHX+0.05% cetyl pyridinium chloride (CPC) without alcohol *versus* placebo) sorted per rinse (with 16 subjects per product) and per follow-up visit (before mechanical debridement and after 3 and 6 months).

Table 2. Detection frequency for specific species (*Streptococcus mutans*, Lactobacilli species, *Porphyromonas gingivalis* and *Prevotella intermedia*) in samples from different niches (supragingival plaque, subgingival plaque for single (Sr) and multi-rooted (Mr) teeth, respectively, and from the saliva) sorted *per* mouth rinse (CHX 0.02% with alcohol, 0.05% CHX+0.05% CPC without alcohol, and placebo) and *per* follow-up visit (baseline and moths 3 and 6)

Sample	Formulation	*S. mutans*			*Lactobaccilli*			*P. gingivalis*			*P. intermedia*		
		month			month			month			month		
		0	3	6	0	3	6	0	3	6	0	3	6
Plaque supra	CHX 0.2%–Al	6	0	0	8	6	5	0	1	0	11	8	8
	CHX 0.05%–CPC 0.05%	10	1	2	13	3	5	3	1	1	10	6	9
	Placebo	3	8	8	11	11	11	4	1	0	13	10	13
Plaque Sub Sr	CHX 0.2%–Al	1	0	0	9	2	3	8	2	5	14	5	5
	CHX 0.05%–CPC 0.05%	6	1	1	10	3	4	10	3	2	12	7	8
	Placebo	3	4	6	9	4	9	5	3	2	14	11	10
Plaque Sub Mr	CHX 0.2%–Al	6	0	0	8	5	8	6	2	4	12	9	9
	CHX 0.05%–CPC 0.05%	7	2	1	14	6	8	11	6	4	15	11	10
	Placebo	4	6	7	10	7	10	6	3	3	13	13	13
Saliva	CHX 0.2%–Al	5	1	1	10	8	11	2	0	1	13	4	5
	CHX 0.05%–CPC 0.05%	12	3	2	14	6	8	6	0	0	10	5	4
	Placebo	4	10	10	10	9	12	5	1	0	14	11	15

Streptococcus sobrinus and *Actinobacillus actinomycetemcomitans* were only infrequently detected.
CHX, chlorhexidine; CPC, cetyl pyridinium chloride; Al, alcohol.

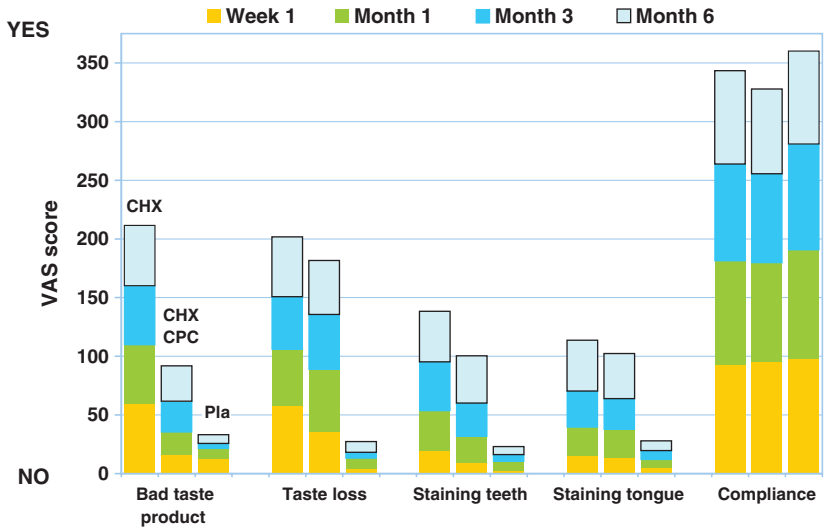

Fig. 6. Subjective ratings (visual analogue ranging from 0 to 100 on anonymous questionnaire) concerning: taste of the rinse, change in taste perception, staining of teeth and tongue, and compliance (with 0 = optimal taste, no change in taste, no staining, or no compliance and 100 = unacceptable taste, dramatic change in taste perception, strong staining and optimal compliance) over a 6-month follow-up period of daily plaque control in combination with twice daily use of a mouth rinse (0.2% chlorhexidine+alcohol (CHX–Al) *versus* 0.05% CHX+0.05% cetyl pyridinium chloride (CPC) without alcohol *versus* placebo) sorted per rinse (with 16 subjects per product) and per follow-up visit (1 week after mechanical debridement, and after 1, 3 and 6 months).

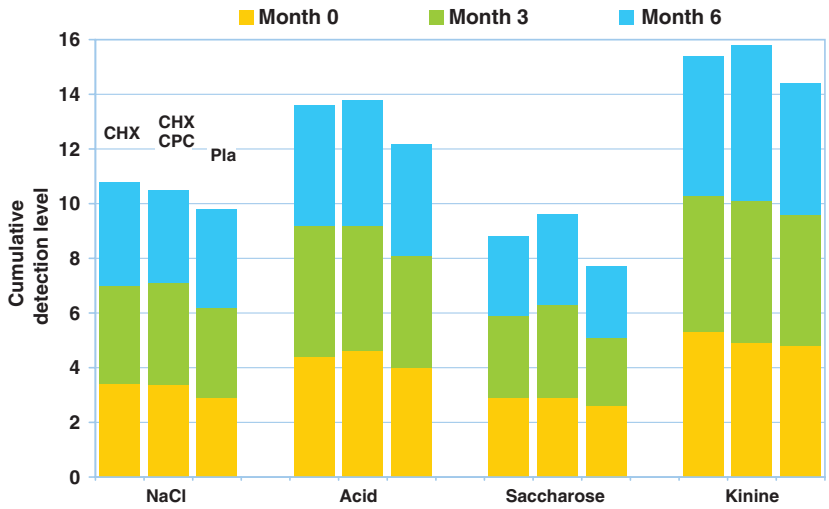

Fig. 7. Cumulative value of minimum concentration before detection of different tastes over a 6-month follow-up period of daily plaque control in combination with twice daily use of a mouth rinse (0.2% chlorhexidine (CHX) +alcohol *versus* 0.05% CHX+0.05% cetyl pyridinium chloride (CPC) without alcohol *versus* placebo) sorted per rinse (with 16 subjects per product) and per follow-up visit (before mechanical debridement and after 3 and 6 months).

Table 3. Mean daily amount of mouth rinse CHX used during the entire study

Product	Consumption/day in ml mean	CHX consumption/day mean
CHX 0.2%–Al	10 ml × 2	20 mg × 2
CHX 0.05%+CPC 0.05%	15 ml × 2	7.5 mg × 2
Placebo	15 ml × 2	0 mg

CHX, chlorhexidine; Al, alcohol; CPC, cetyl pyridinium chloride.

formation (over a period of 11 days), the CHX 0.12%+CPC 0.05% formulation was found to be significantly superior to a CHX 0.12% with 0.05% sodium fluoride, and equally efficient as a 0.2% CHX+alcohol solution (Quirynen et al. 2001).

These data should be interpreted with some caution. Because the molecular weight of CPC is 340 and that of CHX is 896, the number of active molecules in a 0.05% CPC+0.05% CHX solution is high, with 2.6 times more molecules of CPC than of CHX. The latter indicates that when a 0.05% CPC+0.05% CHX solution is compared with a 0.20% CHX solution, the number of active molecules is nearly identical (in the 0.05% CPC+0.05% CHX solution there are 1.470 mmoles of CPC and 0.558 mmoles CHX, thus a total of 2.028 mmoles of ''active'' molecules *versus* 2.232 mmoles in the 0.2% CHX solution). Taking into account that 15 ml of CHX 0.05%+CPC 0.05% had to be used (0.03043 mmoles of active molecules) *versus* 10 ml of the CHX 0.2% solution (0.02232 mmoles), it becomes immediately more understandable why the new formulation (with still 37.5% CHX of the 0.20% CHX solution, see Table 3) is so efficient, especially because for both products a dose-dependent antiplaque effect has been shown (Gjermo et al. 1970, Jenkins et al. 1994a, b).

In in vitro experiments, CPC 0.05% and CHX 0.2% were essentially similar in their antibacterial activity (Roberts & Addy 1981), with even superior fungicidal properties for CPC 0.05% (Giuliana et al. 1997). In vivo however, CPC alone was found to be considerably less effective in reducing plaque (Gjermo et al. 1970, Roberts & Addy 1981, Moran et al. 1994, Renton-Harper et al. 1996, Moran et al. 2000). The latter indicates that the intra-oral substantivity of CPC, is less than for CHX as proved by several other reports (Jenkins et al. 1994c, Elworthy et al. 1996, Young et al. 2003). This is supported by the observation that the antiplaque action of CPC could be increased to that of CHX by doubling the frequency of rinsing (Bonesvoll & Gjermo 1978). The incorporation of CPC in a supragingival slow-release device did, however, not improve the efficacy of CPC (Vandekerckhove et al. 1995). An increase in dosage of CPC seems not advisable because of the risk for severe side effects (Jenkins et al. 1994a).

Studies from the 1960s on the mode of action of CHX also revealed that CHX (as a bi-cationic) accumulates on oral surfaces in aggregates, which are speculated to provide a slow and long-lasting release of the agent into the oral cavity (Hugo & Longworth 1965). CPC, because of its mono-cationic nature, is believed to lose its antibacterial activity as they become rapidly desorbed from the bacterial membrane or other oral sites (Bonesvoll & Gjermo 1978, Moran et al. 1988). The elimination of the ethanol together with the reduction in the concentration of CHX (from 0.20% to 0.05%) had also some beneficial effects on the side effects of CHX. The new formulation had a significant better taste. Also the degree of staining (both scored by the periodontist and by the patient) was slightly less with the new formulation. The improved taste of the new formulation is also not well understood. One can only speculate that the alcohol in the other formulations increased the taste disturbing effect of CHX, and/or was responsible itself for a change in taste perception.

The absence of significant changes in taste perception, when evaluated in a standardized manner was somewhat surprising. This contrasts with previous observations for CHX mouth rinses (Helms et al. 1995), as well as with patients own subjective evaluation (Quirynen et al. 2001). This discrepancy probably indicates that patient's global perception differs from what one can record with a taste recognition test. Another confusing aspect might be the fact that patients in this study also started to clean their tongue, thereby reducing the coating and thus should have an increased perception for some taste (Hyde et al. 1981, Winkler et al. 1999, Quirynen et al. 2003).

So far, only a few studies investigated changes in detection frequency/relative proportion of cariogenic species during periodontal therapy. A first pilot study (Quirynen et al. 1999b) followed 10 patients with severe periodontitis up to 8 months after thorough scaling and root planing in combination with optimal plaque control, and reported a clear increase in the number of *S. mutans*, especially at month 8. In a cross-sectional study form, van der Reijden et al. (2001) subgingival plaque samples from 154 consecutive adult periodontitis patients were tested for the presence and levels of mutans streptococci and putative periodontal pathogens. They divided the patients into four groups based on the stage of periodontal treatment: untreated, after initial periodontal therapy, maintenance phase without periodontal surgery, and finally a group of patients after periodontal surgery. The prevalence of mutans streptococci in the four study groups varied from 82% in the untreated patients, 88% in the group after initial therapy, to 94% in maintenance groups. The mean proportion of mutans streptococci was 6.7% in maintenance patients *versus* 1.9% in untreated patients ($p = 0.005$) and 2.5% in patients after scaling and root planing ($p = 0.04$). This paper (Table 2), together with the preliminary data of another study from our department again supports the idea of a shift from a periopathogenic flora towards a more cariogenic flora after mechanical debridement. It is still unclear how to understand this microbiological shift towards a more cariogenic species. The following aspects may play a role: subgingival overgrowth by *S. mutans* occupying open spots that became available after periodontal therapy (e.g. increased number of free adhesion/receptor sites), a down-growth of *S. mutans* from the supragingival area where it could easily adhere and/or easily survive in the saliva, the creation of a new ecosystem in the subgingival area (anaerobisme, redox potential, pH, nutrition, etc.) that allows/facilitates the growth of *S. mutans,* or a reduced immune reaction (after successful periodontal therapy) to which *S. mutans* was very susceptible. This shift can be prevented via the use of an antiseptic (either containing CHX or amine/stannous fluoride) as adjunct to optimal plaque control.

Conclusion

Within the limitations of the protocol, this long-term study indicates that the new CHX 0.05%+CPC 0.05% solution has an antiplaque effect comparable with that of a 0.2% CHX+alcohol solution, but with less side effects. The absence of alcohol offers some small additional advantages. It is less irritating for the soft tissues, which can be useful for patients with mucositis (after radiotherapy or chemotherapy) or with recurrent oral ulcerations. The new formulation showed a better taste perception and thereby might increase compliance.

Acknowledgements

This study was partially supported by a grant from Dentaid SL, Barcelona, Spain.

References

Addy, M. (1986) Chlorhexidine compared with other locally delivered antimicrobials. A short review. *Journal of Clinical Periodontology* **13**, 957–964.

Addy, M., al Arrayed, F. & Moran, J. (1991a) The use of an oxidising mouthwash to reduce staining associated with chlorhexidine. Studies in vitro and in vivo. *Journal of Clinical Periodontology* **18**, 267–271.

Addy, M. & Moran, J. M. (1997a) Clinical indications for the use of chemical adjuncts to plaque control: chlorhexidine formulations. *Periodontology 2000* **15**, 52–54.

Addy, M. & Moran, J. M. (1997b) Evaluation of oral hygiene products: science is true; don't be misled by the facts. *Periodontology 2000* **15**, 40–51.

Addy, M., Moran, J. & Wade, W. (1994) Chemical plaque control in the prevention of gingivitis and periodontitis. In: Lang, N. P. & Karring, T. (eds). *Proceedings of the 1st European Workshop on Periodontology*, pp. 244–257. Quintessence: Berlin.

Addy, M., Wade, W. & Goodfield, S. (1991b) Staining and antimicrobial properties in vitro of some chlorhexidine formulations. *Clinical Preventive Dentistry* **13**, 13–17.

Alsina, M., Olle, E. & Frias, J. (2001) Improved, low-cost selective culture medium for *Actinobacillus actinomycetemcomitans*. *Journal of Clinical Microbiology* **39**, 509–513.

Axelsson, P. & Lindhe, J. (1981a) Effect of controlled oral hygiene procedures on caries and periodontal disease in adults. Results after 6 years. *Journal of Clinical Periodontology* **8**, 239–248.

Axelsson, P. & Lindhe, J. (1981b) Effect of oral hygiene instruction and professional tooth-cleaning on caries and gingivitis in school-children. *Community of Dental and Oral Epidemiology* **9**, 251–255.

Axelsson, P., Lindhe, J. & Nystrom, B. (1991) On the prevention of caries and periodontal disease. Results of a 15-year longitudinal study in adults. *Journal of Clinical Periodontology* **18**, 182–189.

Barkvoll, P., Rolla, G. & Svendsen, K. (1989) Interaction between chlorhexidine digluconate and sodium lauryl sulfate in vivo. *Journal of Clinical Periodontology* **16**, 593–595.

Bolanowski, S. J., Gescheider, G. A. & Sutton, S. V. (1995) Relationship between oral pain and ethanol concentration in mouthrinses. *Journal of Periodontal Research* **30**, 192–197.

Bollen, C. M. & Quirynen, M. (1996) Microbiological response to mechanical treatment in combination with adjunctive therapy. A

review of the literature. *Journal of Periodontology* **67**, 1143–1158.

Bonesvoll, P. & Gjermo, P. (1978) A comparision between chlorhexidine and some quaternary ammonium compounds with regard to retention, salivary concentration and plaque-inhibiting effect in the human mouth after mouth rinses. *Archives of Oral Biology* **23**, 289–294.

Bonesvoll, P., Lokken, P. & Rolla, G. (1974) Influence of concentration, time, temperature and pH on the retention of chlorhexidine in the human oral cavity after mouth rinses. *Archives of Oral Biology* **19**, 1025–1029.

Checchi, L., Montevecchi, M., Gatto, M. R. & Trombelli, L. (2002) Retrospective study of tooth loss in 92 treated periodontal patients. *Journal of Clinical Periodontology* **29**, 651–656.

Dahlen, G., Lindhe, J., Sato, K., Hanamura, H. & Okamoto, H. (1992) The effect of supragingival plaque control on the subgingival microbiota in subjects with periodontal disease. *Journal of Clinical Periodontology* **19**, 802–809.

Eldridge, K. R., Finnie, S. F., Stephens, J. A., Mauad, A. M., Munoz, C. A. & Kettering, J. D. (1998) Efficacy of an alcohol-free chlorhexidine mouthrinse as an antimicrobial agent. *Journal of Prosthetic Dentistry* **80**, 685–690.

Elmore, J. G. & Horwitz, R. I. (1995) Oral cancer and mouthwash use: evaluation of the epidemiologic evidence. *Otolaryngology and Head and Neck Surgery* **113**, 253–261.

Elworthy, A., Greenman, J., Doherty, F. M., Newcombe, R. G. & Addy, M. (1996) The substantivity of a number of oral hygiene products determined by the duration of effects on salivary bacteria. *Journal of Periodontology* **67**, 572–576.

Ernst, C. P., Prockl, K. & Willershausen, B. (1998) The effectiveness and side effects of 0.1% and 0.2% chlorhexidine mouthrinses: a clinical study. *Quintessence International* **29**, 443–448.

Flotra, L., Gjermo, P., Rolla, G. & Waerhaug, J. (1971) Side effects of chlorhexidine mouth washes. *Scandinavian Journal of Dental Research* **79**, 119–125.

Giuliana, G., Pizzo, G., Milici, M. E., Musotto, G. C. & Giangreco, R. (1997) In vitro antifungal properties of mouthrinses containing antimicrobial agents. *Journal of Periodontology* **68**, 729–733.

Gjermo, P., Baastad, K. L. & Rolla, G. (1970) The plaque-inhibiting capacity of 11 antibacterial compounds. *Journal of Periodontal Research* **5**, 102–109.

Hammond, B. F. (1988) A selective/differential medium for *Wollinella recta*. *Journal of Dental Research* **67**, 327.

Hancock, E. B. & Wirthlin, M. R. Jr (1977) An evaluation of the navy periodontal screening examination. *Journal of Periodontology* **48**, 63–66.

Hellstrom, M. K., Ramberg, P., Krok, L. & Lindhe, J. (1996) The effect of supragingival plaque control on the subgingival microflora in human periodontitis. *Journal of Clinical Periodontology* **23**, 934–940.

Helms, J. A., Della-Fera, M. A., Mott, A. E. & Frank, M. E. (1995) Effects of chlorhexidine on human taste perception. *Archives of Oral Biology* **40**, 913–920.

Herrera, D., Roldan, S., Santacruz, I., Santos, S., Masdevall, M. & Sanz, M. (2003) Differences in antimicrobial activity of four commercial 0.12% chlorhexidine mouthrinse formulations: an in vitro contact test and salivary bacterial counts study. *Journal of Clinical Periodontology* **30**, 307–314.

Hugo, W. B. & Longworth, A. R. (1965) Cytological aspects of the mode of action of chlorhexidine diacetate. *The Journal of Pharmacy and Pharmacology* **17**, 28–32.

Hyde, R. J., Feller, R. P. & Sharon, I. M. (1981) Tongue brushing, dentifrice, and age effects on taste and smell. *Journal of Dental Research* **60**, 1730–1734.

Jenkins, S., Addy, M. & Newcombe, R. G. (1994a) A comparison of cetylpyridinium chloride, triclosan and chlorhexidine mouthrinse formulations for effects on plaque regrowth. *Journal of Clinical Periodontology* **21**, 441–444.

Jenkins, S., Addy, M. & Newcombe, R. G. (1994b) Dose response of chlorhexidine against plaque and comparison with triclosan. *Journal of Clinical Periodontology* **21**, 250–255.

Jenkins, S., Addy, M., Wade, W. & Newcombe, R. G. (1994c) The magnitude and duration of the effects of some mouthrinse products on salivary bacterial counts. *Journal of Clinical Periodontology* **21**, 397–401.

Jones, C. G. (1997) Chlorhexidine: is it still the gold standard? *Periodontology 2000* **15**, 55–62.

Leyes Borrajo, J. L., Garcia, V. L., Lopez, C. G., Rodriguez-Nunez, I., Garcia, F. M. & Gallas, T. M. (2002) Efficacy of chlorhexidine mouthrinses with and without alcohol: a clinical study. *Journal of Periodontology* **73**, 317–321.

Lindhe, J. & Nyman, S. (1975) The effect of plaque control and surgical pocket elimination on the establishment and maintenance of periodontal health. A longitudinal study of periodontal therapy in cases of advanced disease. *Journal of Clinical Periodontology* **2**, 67–79.

Lindhe, J., Westfelt, E., Nyman, S., Socransky, S. S. & Haffajee, A. D. (1984) Long-term effect of surgical/non-surgical treatment of periodontal disease. *Journal of Clinical Periodontology* **11**, 448–458.

Lindhe, J., Westfelt, E., Nyman, S., Socransky, S. S., Heijl, L. & Bratthall, G. (1982) Healing following surgical/non-surgical treatment of periodontal disease. A clinical study. *Journal of Clinical Periodontology* **9**, 115–128.

Loe, H., Schiott, C. R., Karring, G. & Karring, T. (1976) Two years oral use of chlorhexidine in man. I. General design and clinical effects. *Journal of Periodontal Research* **11**, 135–144.

Marks, R. G., Magnusson, I., Taylor, M., Clouser, B., Maruniak, J. & Clark, W. B. (1993) Evaluation of reliability and reproducibility of dental indices. *Journal of Clinical Periodontology* **20**, 54–58.

Mongardini, C., van Steenberghe, D., Dekeyser, C. & Quirynen, M. (1999) One stage full-versus partial-mouth disinfection in the treatment of chronic adult or generalized early-onset periodontitis. I. Long-term clinical observations. *Journal of Periodontology* **70**, 632–645.

Moran, J., Addy, M., Jackson, R. & Newcombe, R. G. (2000) Comparative effects of quaternary ammonium mouthrinses on 4-day plaque regrowth. *Journal of Clinical Periodontology* **27**, 37–40.

Moran, J., Addy, M., Kohut, B., Hovliaras, C. A. & Newcombe, R. G. (1994) Efficacy of mouthrinses in inhibiting the development of supragingival plaque over a 4-day period of no oral hygiene. *Journal of Periodontology* **65**, 904–907.

Moran, J., Addy, M. & Newcombe, R. A. (1988) A clinical trial to assess the efficacy of sanguinarine–zinc mouthrinse (Veadent) compared with chlorhexidine mouthrinse (Corsodyl). *Journal of Clinical Periodontology* **15**, 612–616.

Muhlemann, H. R. & Son, S. (1971) Gingival sulcus bleeding – a leading symptom in initial gingivitis. *Helvetica Odontologica Acta* **15**, 107–113.

Newbrun, E. (1996) Indices to measure gingival bleeding. *Journal of Periodontology* **67**, 555–561.

Nordbo, H., Eriksen, H. M., Rolla, G., Attramadal, A. & Solheim, H. (1982) Iron staining of the acquired enamel pellicle after exposure to tannic acid or chlorhexidine: preliminary report. *Scandinavian Journal of Dental Research* **90**, 117–123.

Nyman, S., Rosling, B. & Lindhe, J. (1975) Effect of professional tooth cleaning on healing after periodontal surgery. *Journal of Clinical Periodontology* **2**, 80–86.

Quirynen, M., Avontroodt, P., Peeters, W., Pauwels, M., Coucke, W. & van Steenberghe, D. (2001) Effect of different chlorhexidine formulations in mouthrinses on de novo plaque formation. *Journal of Clinical Periodontology* **28**, 1127–1136.

Quirynen, M., Avontroodt, P., Soers, C., Zhao, H., Pauwels, M. & van Steenberghe, D. (2003) Impact of tongue cleansers on microbial load and taste. *Journal of Clinical Periodontology*.

Quirynen, M., Gizani, S., Mongardini, C., Declerck, D., Vinckier, F. & van Steenberghe, D. (1999b) The effect of periodontal therapy on the number of cariogenic bacteria in different intra-oral niches. *Journal of Clinical Periodontology* **26**, 322–327.

Quirynen, M., Mongardini, C., Pauwels, M., Bollen, C. M., Van Eldere, J. & van Steenberghe, D. (1999a) One stage full-versus partial-mouth disinfection in the treatment of chronic adult or generalized early-onset periodontitis. II. Long-term impact on microbial load. *Journal of Periodontology* **70**, 646–656.

Rams, T. E., Oler, J., Listgarten, M. A. & Slots, J. (1993) Utility of Ramfjord index teeth to assess periodontal disease progression in longitudinal studies. *Journal of Clinical Periodontology* **20**, 147–150.

van der Reijden, W. A., Dellemijn-Kippuw, N., Stijne-van Nes, A. M., de Soet, J. J. & van Winkelhoff, A. J. (2001) Mutans streptococci in subgingival plaque of treated and untreated patients with periodontitis. *Journal of Clinical Periodontology* **28**, 686–691.

Renton-Harper, P., Addy, M., Moran, J., Doherty, F. M. & Newcombe, R. G. (1996) A comparison of chlorhexidine, cetylpyridinium chloride, triclosan, and C31G mouthrinse products for plaque inhibition. *Journal of Periodontology* **67**, 486–489.

Roberts, W. R. & Addy, M. (1981) Comparison of the in vivo and in vitro antibacterial properties of antiseptic mouthrinses containing chlorhexidine, alexidine, cetyl pyridinium chloride and hexetidine. Relevance to mode of action. *Journal of Clinical Periodontology* **8**, 295–310.

Rogosa, M., Mitchell, J. A. & Wiseman, R. F. (1951) A selective medium for the isolation and enumeration of oral lactobacilli. *Journal of Clinical Periodontology* **30**, 682–689.

Rosling, B., Nyman, S. & Lindhe, J. (1976) The effect of systematic plaque control on bone regeneration in infrabony pockets. *Journal of Clinical Periodontology* **3**, 38–53.

Saxer, U. P. & Muhlemann, H. R. (1975) [Motivation and education]. *Schweizerische Monatsschrift für Zahnheilkunde* **85**, 905–919.

Schupbach, P., Osterwalder, V. & Guggenheim, B. (1995) Human root caries: microbiota in plaque covering sound, carious and arrested carious root surfaces. *Caries Research* **29**, 382–395.

Shapiro, S., Castellana, J. V. & Sprafka, J. M. (1996) Alcohol-containing mouthwashes and oropharyngeal cancer: a spurious association due to underascertainment of confounders? *American Journal of Epidemiology* **144**, 1091–1095.

Silness, J. & Roynstrand, T. (1988) Partial mouth recording of plaque, gingivitis and probing depth in adolescents. *Journal of Clinical Periodontology* **15**, 189–192.

Sissons, C. H., Wong, L. & Cutress, T. W. (1996) Inhibition by ethanol of the growth of biofilm and dispersed microcosm dental plaques. *Archives of Oral Biology* **41**, 27–34.

Smigel, K. (1991) High-alcohol mouthwashes are under scrutiny. *Journal of the National Cancer Institute* **83**, 751.

Smith, R. G., Moran, J., Addy, M., Doherty, F. & Newcombe, R. G. (1995) Comparative staining in vitro and plaque inhibitory properties in vivo of 0.12% and 0.2% chlorhexidine mouthrinses. *Journal of Clinical Periodontology* **22**, 613–617.

Socransky, S. S. & Haffajee, A. D. (2002) Dental biofilms: difficult therapeutic targets. *Periodontology 2000* **28**, 12–55.

Socransky, S. S., Smith, C. & Haffajee, A. D. (2002) Subgingival microbial profiles in refractory periodontal disease. *Journal of Clinical Periodontology* **29**, 260–268.

Syed, S. A. & Loesche, W. J. (1972) Survival of human dental plaque flora in various transport media. *Applied Microbiology* **24**, 638–644.

Turesky, S., Gilmore, N. D. & Glickman, I. (1970) Reduced plaque formation by the chloromethyl analogue of victamine C. *Journal of Periodontology* **41**, 41–43.

Umeda, M., Contreras, A., Chen, C., Bakker, I. & Slots, J. (1998) The utility of whole saliva to detect the oral presence of periodontopathic bacteria. *Journal of Periodontology* **69**, 828–833.

Vandekerckhove, B. N., van Steenberghe, D., Tricio, J., Rosenberg, D. & Encarnacion, M. (1995) Efficacy on supragingival plaque control of cetylpyridinium chloride in a slow-release dosage form. *Journal of Clinical Periodontology* **22**, 824–829.

Walker, C. B., Ratliff, D., Muller, D., Mandell, R. & Socransky, S. S. (1979) Medium for selective isolation of *Fusobacterium nucleatum* from human periodontal pockets. *Journal of Clinical Microbiology* **10**, 844–849.

Westfelt, E., Rylander, H., Dahlen, G. & Lindhe, J. (1998) The effect of supragingival plaque control on the progression of advanced periodontal disease. *Journal of Clinical Periodontology* **25**, 536–541.

Winkler, S., Garg, A. K., Mekayarajjananonth, T., Bakaeen, L. G. & Khan, E. (1999) Depressed taste and smell in geriatric patients. *Journal of the American Dental Association* **130**, 1759–1765.

Winn, D. M., Blot, W. J., McLaughlin, J. K., Austin, D. F., Greenberg, R. S., Preston-Martin, S., Schoenberg, J. B. & Fraumeni, J. F. Jr (1991) Mouthwash use and oral conditions in the risk of oral and pharyngeal cancer. *Cancer Research* **51**, 3044–3047.

Wynder, E. L., Kabat, G., Rosenberg, S. & Levenstein, M. (1983) Oral cancer and mouthwash use. *Journal of the National Cancer Institute* **70**, 255–260.

Ximenez-Fyvie, L. A., Haffajee, A. D., Som, S., Thompson, M., Torresyap, G. & Socransky, S. S. (2000) The effect of repeated professional supragingival plaque removal on the composition of the supra- and subgingival microbiota. *Journal of Clinical Periodontology* **27**, 637–647.

al Yahfoufi, Z., Mombelli, A., Wicki, A. & Lang, N. P. (1995) The effect of plaque control in subjects with shallow pockets and high prevalence of periodontal pathogens. *Journal of Clinical Periodontology* **22**, 78–84.

Young, A., Jonski, G. & Rolla, G. (2003) Inhibition of orally produced volatile sulfur compounds by zinc, chlorhexidine or cetylpyridinium chloride – effect of concentration. *European Journal of Oral Sciences* **111**, 400–404.

Address:
M. Quirynen
Department of Periodontology
School of Dentistry, Oral Pathology & Maxillo-Facial Surgery U.Z. St. Rafael
Capucijnenvoer 33
B-3000 Leuven
Belgium
E-mail: Marc.Quirynen@med.kuleuven.ac.be