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Differences in efficacy of two commercial 0.2% chlorhexidine mouthrinse solutions: a 4-day plaque re-growth study

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Abstract

Background: The purpose of this clinical cross-over study was to examine the antibacterial and plaque-inhibiting properties of two chlorhexidine solutions compared with a negative control.

Material and Methods: Twenty-one volunteers refrained from all oral hygiene measures, but rinsed instead twice daily with 10 ml of a conventional chlorhexidine solution (0.2%; CHX), a chlorhexidine solution with anti-discolouration system (ADS) (0.2%, alcohol-free chlorhexidine solution (CSP)) or a placebo solution (Pla). Plaque index (PI), plaque area (PA) and bacterial vitality were assessed after 24 h (PI1, vital flora (VF)1) and 96 h (PI2; VF2, PA). After a 10-day wash-out period, a new test cycle was started.

Results: Results for Pla were 0.94, 1.59, 27.4 (PI1, PI2, PA) and 79% and 72% (VF1 and VF2). CSP significantly reduced the parameter PI1, PI2 and PA to 0.67 ($p = 0.012$), 1.0 and 15.7 ($p < 0.001$). VF1 and VF2 (63% and 53%) were not significantly affected. The corresponding figures of CHX were 0.42, 0.43, 6.77, 33 and 16%, which were all significantly lower (all $p < 0.001$). On comparing the two chlorhexidine solutions, CHX showed significantly higher reductions of all parameters.

Conclusion: The results suggest that the 0.2% alcohol-containing solution showed superiority in inhibiting plaque re-growth and reducing bacterial vitality compared with the solution with ADS.

Key words: chlorhexidine; dental plaque; mouthrinses; plaque index

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Numerous orally active antimicrobial substances are intended to prevent or reduce the formation of dental plaque. Chlorhexidine digluconate has proved to be the most effective agent in numerous studies (for a review, see Lang & Brecx 1986, Addy et al. 1994) and is still examined intensely, either combined with other ingredients or as a positive control (Auschill et al. 2005, Claydon et al. 2005, Quirynen et al. 2005, Van Strydonck et al. 2005). Undesirable effects such as taste disturbance, tooth discolouration and mucosal erosions, however, limit the duration of use to just a few weeks (Löe & Schiött 1970, Jones 1997). An important factor for the effectiveness of an antibacterial substance against biofilm plaque is adhesion or adsorption of the active ingredients to the tooth (pellicle, dental hard tissue) and the gingiva (substantivity) for as long as possible. Because of its outstanding substantivity, the active ingredient chlorhexidine has proved to be the most effective agent for reducing plaque and gingivitis (Lang & Brecx 1986, Gaffar et al. 1997, Arweiler et al. 2002). A 0.2% (alcohol-containing) chlorhexidine solution is therefore deemed the gold standard and is generally used in studies as a positive control (Jones 1997).

In recent years, various chlorhexidine solutions have come onto the market promising better tolerance with the same efficacy. Because of some side effects as well as cultural aspects concerning the alcohol content in mouthrinses (burning of the mouth, drying out of oral mucosa, possible potential for carcinogenic effects, softening effect on composite filling materials, banned by Islam; Winn et al. 1991, Llewelyn 1994, Penugonda et al. 1994, Elmore &

Horwitz 1995, Shapiro et al. 1996), most of these solutions are alcohol free. In contrast to this is the fact that according to the manufacturers of alcohol-containing products, chlorhexidine in particular requires a certain alcohol content for stability, preservation and efficacy. Furthermore, at 6–7% the content is far below the critical value of 25% for a greater risk of oral and pharyngeal cancer (Winn et al. 1991).

Besides the lack of alcohol, additional ingredients are usually added in order to improve the properties of established chlorhexidine solutions. These ''new'' solutions have for the most part not been subjected to any clinical trial. The manufacturers cite the efficacy of chlorhexidine, a tried-and-tested active ingredient that has been thoroughly investigated. However, this is only for classic preparations and it is so far only in that form that it is termed the gold standard. Numerous authors point out time and again that an active ingredient alone is no guarantee of efficacy, but that the complex product with all its ingredients is what must be tested for its efficacy (Addy et al. 1990).

A new mouthwash, which contains chlorhexidine and additionally an anti-discolouration system (ADS), promises not only to prevent plaque formation but also to avoid staining. Two agents are claimed to interfere, with a synergizing action, with the mechanisms that cause pigmentation, without reducing antiplaque activity (Bernardi et al. 2004). As numerous studies have found that the propensity of chlorhexidine to produce staining in vitro and in vivo was correlated with its effectiveness against plaque (Addy & Roberts 1981, Addy et al. 1989, Jenkins et al. 1989), non-staining chlorhexidine solutions still have to prove their anti-plaque efficacy in clinical trials. A recent in vitro study using the above-mentioned ADS–chlorhexidine solution showed a staining potential similar to that of a conventional 0.2% (alcohol-containing) chlorhexidine mouthrinse product. The authors therefore believe that the ADS solution should be as effective as well-established chlorhexidine mouthrinse products (Addy et al. 2005).

As there are only limited data concerning the anti-plaque activity of this relatively new product, the aim of the clinical study was therefore to investigate whether this 0.2% chlorhexidine solution with ADS has the same plaque-reducing and antibacterial properties as an established (alcohol-containing) 0.2% chlorhexidine solution (the so-called gold standard; Smith et al. 1995, Jones 1997, Van Strydonck et al. 2005).

The study model chosen was the established model of 4-day plaque regrowth, which has already been used in numerous studies by this and other groups (Addy et al. 1983, Moran et al. 2000, Arweiler et al. 2001, 2002, 2003).

Material and Methods

This study was an observer-blind, prospective, randomized, intra-individual comparative, single-centre clinical study. The design of the study was in keeping with the ICH note for guidance on Good Clinical Practice (CPMP/ICH/135/95; 1997) and the Declaration of Helsinki (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964) and subsequent amendments. The study was not commenced until the approval of the ethics committee of Freiburg University had been obtained (# 3/04).

The antibacterial and plaque-inhibiting effect was determined from the relative change in plaque index (PI), from the percentage plaque area (PA) of the anterior teeth after 4 days and from the percentage share of living cells of the plaque biofilm about 2 h after rinsing (vital flora (VF)) compared with a placebo solution. Also investigated were the safety and tolerability of the mouthrinse solutions (by soft tissue examination and asking for unwished events) and subjective evaluation of the mouthrinses by the volunteers by means of a questionnaire (quality of life) at the end of each test cycle.

Volunteers

Twenty-one volunteers (students and staff from Albert-Ludwigs-University, Freiburg) were included in the study. Exclusion criteria were consumption of antibiotics or other medication in the last 3 months that might interfere with plaque formation, poor oral hygiene (papilla bleeding index (PBI) of more than 40%), less than 20 teeth to be included in the evaluation, crowns or restorations on upper and lower incisors, extensive bridges or prosthetic constructions and braces, known intolerance or allergy to mouthrinses, age below 18 years and pregnancy.

Study design

After careful history taking, written volunteer information and signing of the patient consent form, all the study participants received professional tooth cleaning, a sodium fluoride-containing (sodium lauryl sulphate-free) toothpaste (Sensodyne F) and a toothbrush (Sensodyne medium; both GlaxoSmithKline, Bühl, Germany) that they had to use in a hygiene phase before the study (7–10 days) and during wash-out periods. At the start of each test week, the teeth were again cleaned (PI = 0) and the test products were randomly distributed. The first rinsing (1 min.) of each test week was performed under supervision, the other eight took place at home.

For the following four test days, the volunteers had to refrain from all mechanical oral hygiene measures. The use of dental floss was not permitted. Chewing gum was similarly not allowed. Instead, each volunteer rinsed for 1 min. twice daily (in the morning and evening after eating) with the randomly allocated mouthrinse solution. After each test week, there was a 10-day wash-out period to eliminate the antimicrobial chlorhexidine from the enamel and gingiva, which could potentially be absorbed when using mouthrinse solutions. During this week, the volunteers cleaned their teeth with standardized sodium fluoride-containing toothpaste containing no other antibacterial substances, and they were also allowed to practise inter-dental hygiene.

The use of systemic antibiotics or other antibacterial medications had to be reported and resulted in exclusion from the study. The occurrence of adverse events and side-effects was also recorded at each visit.

On the last day of the study, each test subject received professional tooth cleaning and fluoridation of all teeth.

The total duration of the study was 7–10 days for the hygiene phase and 5 weeks for the three test products for each subject.

Test products

The alcohol-free chlorhexidine test solution contained 0.2% chlorhexidine gluconate (Curasept ADS 220, Curaden AG, Switzerland) and ADS with the two agents sodium metabisulphite and

ascorbic acid. The positive control (Chlorhexamed forte® = German brand name for Corsodyl®, GlaxoSmithKline) contained 0.2% chlorhexidine gluconate plus 7% ethanol. The placebo solution (containing sorbitol, peppermint concentrate and alcohol (14%)) was freshly mixed for each test week and filled into brown bottles, on the cap of which there was an integrated measuring cup with 10 ml graduation.

The toothpastes and toothbrushes (GlaxoSmithKline) for the hygiene and wash-out phases, the chlorhexidine solutions (Chlorhexamed forte® and Curasept ADS 220), were purchased from a pharmacy as ready-for-use preparations.

The bottles were coded and randomly distributed by a balanced cross-over design to the test subjects in the individual test weeks by a laboratory technician not otherwise involved in the study so that neither investigator nor test subject could identify the corresponding product. Decoding was performed after the study had ended.

Clinical evaluation

The clinical parameters recorded on days 1 and 4 of each test phase were PI (PI1 and PI2, after Silness & Loe 1964) and on day 4 the percentage PA of the anterior teeth of the upper and lower jaw after staining the plaque with erythrosine, digital photographing and computer-assisted evaluation. PI was recorded on day 1 (after 24 h) on teeth 17–14 and 47–44 (eight teeth) and on day 4 (after 96 h) on teeth 24–27 and 34–37 (eight teeth), in each case on the buccal side, so as not to interfere with plaque formation and hence recording of parameters on other teeth for the other days.

The PBI (after Saxer & Mühlemann 1975) was recorded on all teeth on days 0 and 4 of each test week to ensure that similarly good gingival conditions were present for each test week.

All measurements were made by one calibrated and experienced examiner.

Microbiological evaluation

On day 1 (after 24 h; VF1), in each case approximately 2 h after the morning rinsing, a supragingival plaque sample was taken from the buccal surfaces of teeth 16 and 46 and on day 4 (after 96 h, VF2) from teeth 26 and 36 with a straight probe (EXS 9; Hu-Friedy® Mfg. Co. Inc., Chicago, IL, USA); the samples were streaked on a microscope slide and further processed in the laboratory using the vital fluorescence technique after Netuschil (1983), as described in Arweiler et al. (2001). After completing the staining reaction (with fluorescein diacetate and ethidium bromide), a cover glass was pressed firmly down onto the sample and the evaluation was performed under a microscope (Axioskop 2 plus; Carl Zeiss Göttingen, Germany). Using a digital camera (AxioCam HRc; Carl Zeiss), four digital pictures of different parts of each sample were stored. The vitality of the plaque sample (VF in %) was determined using an image analysis program to discriminate green (= vital) bacteria and red (= dead) bacteria (KS 300; Carl Zeiss).

Statistical evaluation

For the given input values (a level of significance of $\alpha = 0.05$, a 20% reduction in parameter with a 10% standard deviation as clinically relevant and a sample size of 19 subjects), a power $(1 - \beta)$ of > 0.90 was computed for two-sided null hypothesis H_0 (http://www.stat.ucla.edu/calculators/powercalc/).

After the last test had been completed and the mouthrinse order decoded, the evaluation was performed with the computer program Statistical Package of Social Science/SPSS 11.0. The mean values of the clinical parameters (PI and PA) and the vitality of the supragingival plaque flora were calculated for each rinse solution. PI and vitality were recorded on 2 different days, while for the PA there were only data from 1 day (day 4) owing to the necessary staining. First, analysis of variance (ANOVA) was performed. The normal distribution of the test data of the individual rinse solutions and the differences compared with placebo solution were checked with the Kolmogorov–Smirnov test. As the data were normally distributed, a parametric test was able to be used. Significant differences between the individual rinse solutions and the placebo solution were determined via Student's *t*-test for dependent samples. Bonferroni's adjustments for multiple comparisons were made. Data were analysed for cross-over effects, but no influence of the order of use was found.

Results

Of the 21 test subjects, 19 of them, 13 female and six male with an average age of 29.1 ± 9.8 years (aged between 20 and 52 years), took part until the end of the study. One female subject did not appear again after the screening visit, and in the last test week another subject had to withdraw owing to appendicitis and administration of antibiotics. This event was considered not to be associated with the agents used in the study. No further adverse events occurred.

The PBI, which was recorded at the start (day 0) and at the end of each test cycle (day 4), was considerably below one in all test subjects, thus ensuring that no gingivitis had developed during the rinsing and wash-out phases. There was even a trend towards an improvement in gingival conditions but there were no significant differences between the individual test phases; thus, equal plaque re-growth conditions could be assumed for each rinse solution.

The mean values for the parameters PI1 and PI2, percentage PA and vitality of the plaque biofilm (VF1 and VF2) are given in Table 1.

ANOVA showed a significant influence ($p \leqslant 0.001$) of the solution on all parameters (PI1, PI2, VF1, VF2, PA). Differences between the active solutions and the placebo were tested by means of the paired *t*-test.

The negative control (placebo solution; Pla) attained the highest values both for the plaque parameters (PI1 and PI2 of 0.94 and 1.59; PA 27.35%) and for the microbiological data (VF1 78.6% and VF2 71.74%). The alcohol-free chlorhexidine solution (CSP) had a significant effect on plaque parameters. Compared with the placebo values, it achieved reductions of 29% ($p = 0.12$) and 37% ($p \leqslant 0.001$) in PI1 and PI2 and of 43% ($p \leqslant 0.001$) in PA. The microbiological parameters VF1 (62.66%) and VF2 (53.35%) were not significantly affected by CSP compared with the placebo solution (20% reduction on day 1; 26% reduction on day 4).

CHX achieved reductions of 55% and 73% in PI1 and PI2 compared with the negative control, and a 75% reduction in PA. These reductions were not only significantly different from the placebo solution ($p \leqslant 0.001$), they were also significantly lower than the values of CSP ($p \leqslant 0.01$ for PI1 and PA, $p \leqslant 0.001$ for PI2). In the case of the microbiological parameter ''Vitality of plaque bacteria'', the CHX solution also demonstrated a significant effect. Vitality was reduced by 58% on day 1 and by 78% on day 4 compared with the placebo solution ($p \leqslant 0.001$). The effect of CHX

Table 1. Mean values and statistical comparison between placebo and the two chlorhexidine solutions in terms of (a) plaque index, (b) plaque area and (c) Biofilm vitality

Solution	PI1	*p* (ANOVA)	Red (%)	*p* (*t*-test)	PI2	*p* (ANOVA)	Red (%)	*p* (*t*-test)
(a) Plaque index on day 1 (PI1) and day 4 (PI2)								
Pla	0.94 ± 0.35	⩽0.001			1.59 ± 0.18	⩽0.001		
CSP	0.67 ± 0.33		29	0.012	1.00 ± 0.44		37	⩽0.001
CHX	0.42 ± 0.21		55	⩽0.001	0.43 ± 0.33		73	⩽0.001

Solution	PA	*p* (ANOVA)	Red (%)	*p* (*t*-test)
(b) Plaque area (PA, in %)				
Pla	27.35 ± 12.79	⩽0.001		
CSP	15.72 ± 10.84		43	⩽0.001
CHX	6.77 ± 6.97		75	⩽0.001

Solution	VF1	*p* (ANOVA)	Red (%)	*p* (*t*-test)	VF2	*p* (ANOVA)	Red (%)	*p* (*t*-test)
(c) Biofilm vitality on day 1 (VF1) and day 4 (VF2)								
Pla	78.69 ± 16.22	⩽0.001			71.74 ± 21.70	⩽0.001		
CSP	62.66 ± 24.42		20	NS	53.35 ± 32.80		26	NS
CHX	32.92 ± 27.26		58	⩽0.001	15.52 ± 14.17		78	⩽0.001

Statistical analysis performed by ANOVA (level of significance $p \leqslant 0.05$) and a post-hoc test (by means of a paired *t*-test compared with placebo) with Bonferroni's adjustments.

Pla, placebo solution; CSP, Curasept ADS 220; CHX, chlorhexamed forte; Red, reduction compared with placebo solution in %; *p*, *p*-value of statistical evaluation; NS, not significant.

on plaque bacteria was also significantly better than that of CSP ($p \leqslant 0.001$).

Discussion

The present short-term study model dealt with the clinical and antibacterial efficacy of a relatively new chlorhexidine solution on supragingival plaque growth and bacterial vitality. A placebo solution (negative control) and 0.2% chlorhexidine solution, which was well researched and has proven efficacy in numerous studies (Mendieta et al. 1994, Smith et al. 1995), served as comparison. The study design (4-day plaque re-growth study) has been used in numerous investigations and can be described as an established method for testing the antibacterial and plaque-inhibiting effect of an oral hygiene product (Addy et al. 1983, Arweiler et al. 2001, 2003).

Owing to the side effects of conventional chlorhexidine solutions like yellow-brown or black stains on the back of the tongue or on teeth and burning mouth because of the alcohol content of such solutions, increasingly, manufacturers tended to refrain from introducing an alcohol content in chlorhexidine products while adding other substances which claim to prevent staining reactions. However, data concerning the efficacy of such new products are sparse. In the literature, reference is made time and again to the fact that, on the basis of the simple presence of an active substance, nothing can be said about its effectiveness in the overall combination without testing against a well-researched (alcohol-containing) chlorhexidine solution (Addy et al. 1990).

In the present study, the conventional (alcohol-containing) chlorhexidine solution proved to be the product with the greatest plaque-inhibiting and antibacterial effects. CSP definitely showed a significant effect when compared with the placebo solution, but was unable to match the noticeable plaque inhibition of the CHX solution.

The incline of the PI in the placebo group during the 4 consecutive days as well as the general value of the plaque indices (1.0–1.6) were in accordance with published evidence, as was the significant retardation of the plaque accumulation with CHX (reductions between 50% and 75%; Addy et al. 1983, 1990). The present study showed, like many previous studies, that rinsing with chlorhexidine strongly inhibits plaque re-grwoth in the absence of mechanical hygiene measures (Jones 1997).

The weaker efficacy of CSP against plaque bacteria might be explained by the effect of alcohol on the potential stability and action of chlorhexidine. Recent findings from a clinical study have failed to show differences between an alcohol-free and an alcohol-containing mouthrinse (Leyes Borrajo et al. 2002). However, in the above-mentioned study, patients maintained their oral hygiene measures and brushing habits throughout the investigation and therefore it cannot be excluded that differences between both chlorhexidine solutions might have been overlapped by differences in oral hygiene habits. Furthermore, both chlorhexidine solutions were freshly prepared by the same laboratory and did not represent commercially available products. On the other hand, another study investigating the antimicrobial activity of four commercial chlorhexidine formulations (Herrera et al. 2003) has shown that the one with alcohol was more active than those without alcohol. The results have also indicated that the addition of cetylpyridinium chloride (CPC) to the alcohol-free chlorhexidine formulation could not only compensate for the alcohol but also resulted in an increase in antimicrobial activity.

A more important reason for the weaker antibacterial and anti-plaque activity of CSP is the addition of the ADS to the solution, which ought to reduce the staining potential of chlorhexidine but apparently at the cost of a significant reduction in benefits to plaque control.

ADS may interact with the active ingredient chlorhexidine. The two active

agents, metabisulphite and ascorbic acid, may compete against the chlorhexidine molecule and/or inhibit the adhesion of the positively charged molecule to the tooth substance or other intra-oral structures. This would be in line with findings indicating that a reduction in the tendency to stain may also lead to a loss of plaque inhibition (Claydon et al. 2001, Sheen & Addy 2003). However, in a recent in vitro study, Addy et al. (2005) found that the ADS–chlorhexidine rinse has the same staining potential as a conventional, well-established, alcohol-containing chlorhexidine solution. The authors suppose that this would correlate with a similar anti-plaque efficacy as seen in established chlorhexidine rinse products, but simultaneously they admit that more clinical studies are necessary to confirm this conclusion. It is also possible that in vivo there is, as mentioned above, continuous competition between the ''anti-plaque'' and the ''anti-staining'' ingredients, and that this is responsible for the varying results. The findings of both studies are in contrast to Bernardi et al. (2004), who showed similar anti-plaque efficacy and simultaneously lesser staining compared with a conventional 0.2% chlorhexidine mouthwash, which is not specified. It is possible that in the Bernardi et al. (2004) study, the use of mouth rinses alongside normal oral hygiene could have masked any (positive or negative) effect of a rinse and moreover the term was too short to show an effect on staining.

Altogether, the reductions of CSP are in the range of the reductions of various other mouthrinse products for ''soft chemoprevention'' (30–50%; Arweiler et al. 2001, 2002, 2003). For the purpose of similar conditions, 4-day plaque regrowth studies are performed with healthy volunteers with a high level of oral hygiene. Possibly, the effects of the solutions are higher on volunteers with gingivitis, which is the target group for the use of chlorhexidine products.

The effect of this ADS–chlorhexidine solution measured in the present study, however, was less than that of an established (gold standard) formulation.

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Clinical Relevance

Scientific rationale for the study: New antibacterial products should be clinically tested for their efficacy even if they contain known antibacterial agents. The presence of chlorhexidine is no guarantee of efficacy and therefore should be tested in the complex product with all its ingredients.

Principal findings: The results suggest that the alcohol-containing chlorhexidine solution was superior in inhibiting plaque re-growth and reducing bacterial vitality compared with a relatively new formulation that contained chlorhexidine as well as an additional anti-discolouration system.

Practical implications: If there is no contraindication for the use of alcohol-containing products, an alcohol-containing 0.2% chlorhexidine solution can still be referred to as the gold standard for treatment.