Chlorhexidine and the control of plaque and gingivitis
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Chapter 4

Chlorhexidine mouthrinse in combination with an SLS-containing dentifrice and a dentifrice slurry

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Introduction

Effective plaque control is crucial for the maintenance of periodontal health. For most individuals, the most efficient, safe and economical method of removing plaque, is tooth brushing with a dentifrice. However, for many, a plaque-free dentition, obtained by tooth brushing with dentifrice only, is a difficult goal to achieve. The adjunctive use of an antiseptic agent may therefore be justified. After 3 decades of use in oral medicine, chlorhexidine digluconate (CHX) is still considered as the leading antiseptic to combat biofilms in supragingival and oral mucosal sites (Addy 1986, Addy & Moran 1997).

One of the most widely used detergents in dentifrice is sodium lauryl sulphate (SLS). Unfortunately, in vitro, SLS and CHX may act as antagonists (Rölla et al. 1970, Kirkegaard et al. 1974, Rölla & Melsen 1975, Bonesvoll et al. 1977, Barkvoll et al. 1988). ‘In vivo’, the interactions between CHX and SLS have been studied by Barkvoll et al. (1989) and Owens et al. (1997). They both concluded that CHX and SLS are not compatible in the oral cavity, even when they are introduced separately. Ever since, it has been recommended that the time between a CHX rinsing and tooth brushing with an SLS-containing dentifrice should at least be 30 minutes, probably close to 2 hours, in order to avoid reduction in the anti-microbial effect of CHX. To optimize the efficacy of a CHX rinse, tooth brushing with dentifrice should be suspended or tooth brushing should be performed with dentifrice formulations without antagonistic ingredients (Owens et al. 1997) or without dentifrice. In these initial studies, SLS was used as an aqueous solution (Barkvoll et al. 1989) or as water dentifrice slurry (Owens et al. 1997). The proposed inhibiting effect of SLS has not been tested if one uses this product as one would for daily oral hygiene.

Recently, these practical guidelines have been questioned by Van Strydonck et al. (2004a). In a 4-day plaque accumulation model, the plaque-inhibition of a 0.2% CHX rinse in one jaw was investigated under the influence of tooth brushing with a 1.5% SLS-containing dentifrice in the opposite jaw. On the basis of their clinical results, it appeared that the anti-plaque efficacy of the 0.2% CHX mouth rinse was not reduced.

A second study (Van Strydonck et al. 2004b) with a similar design confirmed the findings of the first study. This time, the study was performed under supervision, the order of rinsing-brushing was reversed, and different brands of dentifrice, with and without SLS, were compared. Again, the results showed that the anti-plaque efficacy of CHX was not reduced by everyday tooth brushing with a dentifrice. Irrespective if the dentifrice contains SLS, or was used before or after the rinse, these two dentifrice studies by Van Strydonck et al. (2004...
a,b) did not support the conclusions of the earlier work (Barkvoll et al., 1989 and Owens et al., 1997).

The most plausible explanation of the conflicting results of Van Strydonck et al. (2004 a, b) compared to the earlier CHX-SLS interaction studies (Barkvoll et al. 1989, Owens et al. 1997) seems to be the use of an SLS-containing dentifrice by Van Strydonck et al. (2004 a, b) instead of an SLS rinse by the other authors.

The aim of the present study was therefore to compare, the plaque-inhibitory effect of a 0.2% CHX rinse when preceded by everyday toothbrushing with a 1.5% SLS-containing dentifrice in the opposite jaw, to the effects of the same rinse when preceded by rinsing with an SLS-containing dentifrice slurry and rinsing with 0.2% alone. The present parallel study intended to eliminate any carry-over effects.

Materials and methods

Subjects

120 subjects, 54 males and 66 females, aged between 16 and 70 (mean age 43) were found to be suitable for the study. Subjects were in good general health without a medical history or medication that might interfere with the outcome of the study. All subjects were dentate with at least 24 scorable teeth, excluding third molars or crowned teeth with porcelain or golden restorations.

They were excluded if they had fixed or removable orthodontic appliances or removable prosthesis, pockets > 5 mm or attachment loss > 2 mm. On approval, all the volunteers received a personal instruction schedule, signed an informed-consent paper and, in order to participate, agreed to the following:

- Products would only be used under supervision of 2 dental assistants twice daily at set times.
- Appointments (days and hours) were strict and could not be changed.
- It was not allowed to perform another form of oral hygiene other than the one assigned.
- It was not allowed to eat, drink or rinse with water within 30 minutes after the rinsing procedure with the test rinse.
- Any change in medical status or medicine intake was to be reported.
The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practice.

**Procedure**

The study had a single-blind, randomized, 3-arm parallel design. It used a 4-day plaque accumulation model to compare 3 groups of healthy volunteers with 3 different, supervised, oral hygiene regimens (A, B and C). The 3 groups (n=40 each) were matched for sex and age.

The experiment was performed in one randomly assigned (upper or lower) jaw, called the “study” jaw. The opposite jaw, referred to as the “dentifrice” jaw, served only to introduce the influence of toothbrushing with a dentifrice on the anti-plaque efficacy of the CHX in the “study” jaw of the same mouth. At the end of the 4-day test period, plaque and gingival bleeding were scored in the study jaw. In all the regimens the oral hygiene procedure was finalized by rinsing with a CHX 0.2% solution for 1 minute. The study jaw was not brushed during the experiment. Regimen A (positive control) consisted of rinsing with 0.2% CHX alone. In regimen B, rinsing with CHX was preceded by rinsing (60 seconds) with a 1.5% SLS-containing slurry, while in regimen C rinsing with 0.2% CHX was preceded by toothbrushing (60 seconds) with a 1.5% SLS-containing dentifrice in the “dentifrice” jaw (table 1). After brushing, the dentifrice foam was thoroughly expectorated and the mouth was shortly (3 seconds) rinsed with water. No other oral hygiene measures were allowed.

At baseline (day 1), all subjects received thorough professional prophylaxis by two subsequent operators. After staining the teeth with an aqueous erythrosine disclosing solution (0.9%), a first operator (Ph.D) removed all supragingival plaque, stain and calculus by sonic airscaler and polished all the teeth. Subsequently, after a second disclosing, the second operator (L.V.D.S.) made sure that all visible remnants of plaque, stain and calculus were removed from the teeth.

Throughout the duration of the study, subjects were asked to refrain from all forms of oral hygiene other than assigned products (e.g. dentifrice, non-study toothbrushes & mouthrinses, floss, woodsticks, interdental brushes, electric toothbrushes, oral irrigators, etc.).

Subsequently, they were randomly assigned to 1 of the 3 regimens. Instructions for the allocated regimen were given to each subject by a dental assistant, who also supervised the further conduct of the study.

During the test week, the subjects were performing their given test regimes, while two dental assistants supervised the brushing-rinsing twice daily at set times, during the
study duration. After 4 days of undisturbed plaque accumulation, the amount of plaque was scored in the “study” jaw. After disclosing the teeth with an erythrosine solution, plaque was assessed at 6 sites around each tooth, according to the modifications of Turesky et al. (1970) and Lobene et al. (1982) of the Quigley & Hein (1962) plaque index. In addition, at day 4, bleeding was scored in the “study” jaw by the use of a WHO approved ball ended probe (Ash Probe EN15) and assessed by bleeding on marginal probing (Van der Weijden et al.1994, Lie et al. 2001). In short: the marginal gingivae were probed at an angle of approximately 60° to the longitudinal axis of the tooth. The bleeding was assessed at six sites per tooth. The gingival units which bled upon probing were recorded (score 0, 1 and 2) giving non-bleeding sites a (0), pin prick bleeding sites a (1) and excess bleeding sites a (2). Bleeding was scored within 30 seconds after probing. After the test, subjects resumed to their normal tooth cleaning habits. All clinical measurements were performed under the same conditions by one and the same, well-trained, examiner (D. V. S.), who was blinded to the treatment.

<table>
<thead>
<tr>
<th>Table 1. Regimens</th>
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| Regimen A | Rinsing only with 0.2% CHX rinse for 60 seconds.*  
| | No brushing was allowed. |
| Regimen B | Rinsing with a 3 g/10 ml water SLS-containing dentifrice slurry for 60 seconds.†  
| | Expectoration of the slurry, not followed by rinsing with water.  
| | Rinsing with a 0.2% CHX rinse for 60 seconds.*  
| | No brushing was allowed. |
| Regimen C | Toothbrushing in the “dentifrice” jaw with 1 cm of a 1.5% SLS-containing dentifrice for 60 seconds.‡  
| | Expectoration of remaning dentifrice, followed by rinsing with water for 3 seconds.  
| | Rinsing with 0.2% CHX rinse for 60 seconds.*  
| | No brushing of the study jaw was allowed. |

CHX, chlorhexidine digluconate; SLS, sodium lauryl sulphate.  
* Corsodyl®, GlaxoSmithKline (GSK), Zeist, the Netherlands).  
† Aquafresh Regular® slurry (GSK) contains 3 g dentifrice Aquafresh Regular® (GSK)/10 ml water solution.  
‡ Aquafresh Regular® (GSK) dentifrice contains 1.5% SLS, pyrophosphate, 0.24% NaF (0.3% NaF = 1500 p.p.m.).
Data analysis
Full mouth mean plaque and bleeding scores were calculated. Plaque scores were considered as the primary outcome variable. Kruskall Wallis tests were used to test for differences between the 3 regimens. Mann Whitney tests were used for post-testing. The $p$-values were adjusted for multiple testing using Bonferoni corrections (a factor 3 was used since in total 3 tests were performed). Furthermore 95% confidence intervals were calculated for differences between the groups. $p$-values < 0.05 were considered as statistically significant. Power-calculations show that the study design was able to discern a difference of 0.35 with a N of 40 and a pooled standard deviation of 0.56 at a power of 80%.

Results
119 of the selected subjects (n=120) completed the study without protocol violation. One subject was withdrawn from the trial because of non-compliance with the study protocol and was not included in the analysis. No adverse effects were noted. Table 2 shows the mean plaque-scores of the “study” jaws for the 3 regimens. The mean plaque index for the “CHX rinsing only” regimen (A), was 1.17, for the “Slurry-CHX” regimen (B) 1.62 and for the “brushing-CHX” regimen (C) 1.14. Analysis showed a significant difference between the 3 regimens ($p$-value = 0.006). Explorative testing revealed a higher plaque index for the “Slurry-CHX” as compared to each of the other 2 regimens (Table 3). The mean bleeding index in the “study” jaw for regimen (A), was 0.24, for regimen (B) was 0.18 and for (C) was 0.20 (Table 2). There was no significant difference in bleeding on marginal probing score between the 3 different regimens (Table 4).

<table>
<thead>
<tr>
<th></th>
<th>CHX alone (n = 40)</th>
<th>Slurry-CHX (n = 40)</th>
<th>Dentifrice-CHX (n = 39)</th>
<th>$p$-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque index</td>
<td>1.17 (0.62)</td>
<td>1.62 (0.55)</td>
<td>1.14 (0.51)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Bleeding index</td>
<td>0.24 (0.17)</td>
<td>0.18 (0.15)</td>
<td>0.20 (0.17)</td>
<td>0.2842</td>
</tr>
</tbody>
</table>

CHX, chlorhexidine digluconate; SLS, sodium lauryl sulphate.
*Kruskall-Wallis test.
Table 3. *p*-values of post-testing* and 95% confidence intervals for differences in plaque indices between regimens

<table>
<thead>
<tr>
<th>Regimens</th>
<th>Original p-values (Mann-Whitney test)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B</td>
<td>0.0027*</td>
<td>0.20-0.72</td>
</tr>
<tr>
<td>A-C</td>
<td>0.8947</td>
<td>-0.23-0.28</td>
</tr>
<tr>
<td>B-C</td>
<td>0.0002*</td>
<td>0.25-0.72</td>
</tr>
</tbody>
</table>

Regimen A: CHX alone.
Regimen B: Slurry-CHX.
Regimen C: Dentifrice-CHX.

CHX, chlorhexidine digluconate; SLS, sodium lauryl sulphate.

*Significant after Bonferroni’s corrections (factor 3) for multiple comparisons.

Table 4. *p*-values of post-testing* and 95% confidence intervals for differences in marginal bleeding indices between regimens

<table>
<thead>
<tr>
<th>Regimens</th>
<th>Original p-values (Mann-Whitney test)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B</td>
<td>0.1018*</td>
<td>-0.12-0.018</td>
</tr>
<tr>
<td>A-C</td>
<td>0.6948</td>
<td>-0.04-0.11</td>
</tr>
<tr>
<td>B-C</td>
<td>0.3339*</td>
<td>-0.09-0.05</td>
</tr>
</tbody>
</table>

Regimen A: CHX alone.
Regimen B: Slurry-CHX.
Regimen C: dentifrice-CHX.

CHX, chlorhexidine digluconate; SLS, sodium lauryl sulphate.

*Significant after Bonferroni’s corrections (factor 3) for multiple comparisons.
Discussion

Dentifrice ingredients such as SLS have been shown to inhibit the activity of CHX (Barkvoll et al. 1989, Owens et al. 1997). Barkvoll et al. (1989) allowed their subjects to rinse with an aqueous solution of 0.2% SLS. In the study of Owens et al. (1997) the dentifrice, being a sodium fluoride and sodium monofluorophosphate SLS containing product, was made into a 3g/10 ml water slurry. In both these studies on CHX-SLS interaction, the oral cavity was not cleared from SLS before the rinsing with CHX. In previous dentifrice studies by Van Strydonck et al. (2004a,b), and also in the present study, the SLS detergent was tested in an “everyday oral hygiene” situation, i.e. toothbrushing with an SLS-containing dentifrice. As in daily life, the panelists expectorated the remnants of dentifrice and rinsed with water immediately after the brushing with the dentifrice. This cleared the oral cavity of the residual SLS dentifrice. A lower intra-oral SLS-concentration and a shorter contact time of SLS with CHX, is considered to be responsible for the observed absence of reduction in plaque inhibition when using a CHX rinse in combination with dentifrice (Van Strydonck et al. 2004a,b). This supposition has been confirmed in the present study. Compared to the CHX-alone group, the dentifrice group showed no reduced plaque inhibition, while the slurry group, which is comparable to earlier studies (Barkvoll et al. 1989, Owens et al. 1997), showed a significantly higher level of plaque accumulation.

Analogous to the studies of Barkvoll et al. (1989) and Owens et al. (1997), both studies by Van Strydonck et al. (2004a,b) used a cross-over model, using each patient as its own control. That design was chosen to provide considerable power to detect differences with relatively small sample sizes. Crossover experiments can yield great savings if the assumption of no carry-over effect is valid (Grizzle 1965, Louis et al. 1984). The 4-day plaque accumulation model includes a thorough dental prophylaxis prior to the commencement of each test regimen. The magnitude and duration of this was not established and frequent prophylaxis may influence the level of gingival health. It has been shown that, with healthy gingival tissues, less plaque develops (Ramberg et al. 1994, 1995). This may introduce an unwanted carry-over effect which could possibly have obscured the interaction that is the subject of the former CHX-SLS interaction studies. The present study was designed to eliminate any possible carry-over effect. For this reason, it has a parallel design.

Several studies have shown that the development of plaque may be dependent on a number of factors such diet (Rateitschak-Pluss & Guggenheim 1982), surface roughness (Quirynen et al. 1990), the periodontal condition (Rowshani et al. 2004, Dahan et al. 2004)
and the bacterial salivary load (Dahan et al. 2004). Hillam & Hull (1977) showed in an experimental gingivitis study that the amount of plaque which developed in 24h in gingival health at baseline was considerably less as compared with the amount of plaque developed in 24h at the end of the experimental gingivitis period. More extensive studies performed by Lang et al. (1973), Breckx et al. (1980), Goh et al. (1986), Quirynen et al. (1991), Ramberg et al. (1994,1995), Daly & Highfield (1996) and Rudiger et al. (2002), all confirmed that the periodontal condition is of foremost importance in the rate of de novo plaque formation. Using 3 separate groups, in the present parallel design, may introduce an unwanted effect as a result of varying levels of gingival health. Therefore, in this study, in addition to plaque levels, the level of gingival health was assessed to make sure that this was not an interfering factor with the outcome of the study. In terms of bleeding on marginal probing, no significant difference was found between the 3 regimens. Table 4 shows the 95% CI for the differences in bleeding scores. These intervals are narrow and ‘0’ is not far from the middle of the interval. It can therefore be concluded that the level of gingival health was not the origin of a more elevated plaque index as observed in the slurry group.

The present study has shown that the anti-plaque efficacy of a 0.2% CHX rinse is reduced under the influence of an SLS-containing dentifrice solution, which is in agreement with the earlier findings of Barkvoll et al. (1989) and Owens et al. (1997). However, their conclusions about the influence of everyday toothbrushing with an SLS-containing dentifrice on the anti-plaque efficacy of a 0.2% CHX may have been premature. Both the results of the present study and the 2 previous studies on efficacy of CHX combined with the interaction of different dentifrices for toothbrushing by Van Strydonck et al. (2004a,b), clearly indicate that the anti-plaque effect of a CHX mouthrinse is not reduced under the influence of normal everyday toothbrushing with a dentifrice, irrespective if the dentifrice contains SLS, or was used before or after the rinse. This observation has a practical implication for periodontal treatment. After periodontal surgery in for instance one quadrant in the mouth, the use of a CHX mouthrinse is frequently subscribed to optimize adequate wound healing of the operated area.

One needs not to be concerned when toothbrushing is performed before or after the rinsing procedure.

In conclusion, when 0.2% CHX rinse was preceded by rinsing with an SLS-containing slurry the anti-plaque efficacy of CHX was reduced. However, when everyday toothbrushing with an SLS-containing dentifrice preceded a 0.2% CHX rinse, there was no significant difference from 0.2% CHX alone.
Acknowledgments

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