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ORIGINAL ARTICLE

Effect of an intensified treatment with 40% chlorhexidine varnish on plaque acidogenicity

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Abstract Previous work showed that a single application of 40% chlorhexidine varnish, EC40®, reduced plaque acidogenicity upon sucrose challenge during less than 3 weeks. It was questioned whether lactic acid production could be reduced significantly longer when the treatment was intensified. Therefore, the effects of three consecutive EC40® applications on plaque acidogenicity were evaluated. Nine subjects who participated in the previous study received three full mouth EC40® applications within 1 week. Before the first application and up to 9 weeks after the third application, plaque samples were taken after a 10% sucrose rinse and analyzed for organic acids with capillary electrophoresis. At baseline, the mean provoked lactic acid concentration was 1.64 (±0.69) μmol/mg protein. At the first and seventh day after the third application, there was too little plaque to measure acid concentrations. At 2 weeks after the third application, lactic acid concentrations were significantly reduced ($p<0.05$). The acid concentrations 3 weeks after the third application (1.61 (±0.99) μmol/mg protein) did not differ from the values at baseline (paired T test, $p>0.05$). We conclude that a triple 40% chlorhexidine varnish treatment did not affect plaque acidogenicity for more than 3 weeks. From comparison with a previous study, we conclude that the triple treatment with EC40® within 1 week was not more effective in reducing plaque acidogenicity than the single one.

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Introduction

Chlorhexidine can be used in addition to fluoride for patients with a high caries risk. As caries is a continuous process, the aid of chlorhexidine would require long-term use. Because of the side effects—such as bad taste and discoloration of the teeth—and the required compliance, long-term daily use of mouth rinses is unrealistic. Therefore, dental varnishes with lasting antimicrobial effects developed to be applied every 3–6 months would be a desirable and suitable alternative [6, 19].

Our previous study on the effect of a single 40% chlorhexidine varnish application showed a suppression of salivary mutans streptococci that was paralleled by a decrease in the acidogenicity of dental plaque. The subsequent regrowth of the salivary mutans streptococci within less than 3 weeks was accompanied by a recovery of the acidogenicity [8].

It is doubtful whether a 3-week period of reduced acidogenicity of dental plaque contributes substantially to the reduction of caries when the treatments are repeated with a 3 to 6 months interval. A more frequent application of chlorhexidine varnish e.g. once every 3 weeks might be a solution but would require a lot of compliance of the participants and would be costly as a caries preventive measure.

For varnishes containing 1–10% chlorhexidine, it was demonstrated that multiple applications within short periods of time prolonged the suppression of salivary [14, 15] and plaque mutans streptococci [2]. For 40% chlorhexidine varnish, a small advantage in the suppression of the number

of mutans streptococci was reported after two applications with a 1-week interval compared to a single application [10, 17]. The clinical importance of the reported suppression of mutans streptococci in time or in numbers can be questioned. Furthermore, the effect on the acidogenicity of dental plaque was not assessed in these studies.

The aim of this study was to evaluate the effects of a triple application of EC40® on acid production in sucrose-challenged plaque.

Materials and methods

Subjects

Six months after the single application study [8], all 13 dental students were invited for this intensified application program. Four students could not participate in this second trial because of scheduling conflicts to the strict design. The remaining 9 subjects (5 men, 4 women, mean age 25 years (±9)) signed an informed consent letter that was reviewed by the scientific board of the dental school. They were all in good general health and showed a fairly good overall level of oral hygiene.

Set up

Two weeks before the chlorhexidine applications, and throughout the study period, the participants brushed their teeth twice a day with regular sodium fluoride toothpaste (Prodent Cool Mint 1,450 ppm F^-, Sara Lee, Veenendaal, The Netherlands). Neither dental hygiene instructions nor professional tooth cleaning were given before the start of the treatment. All participants received three applications of EC40® (Explore, Nijmegen, The Netherlands) every second day within 1 week. At baseline and after the third chlorhexidine application, sucrose-challenged plaque was sampled as described below at various time intervals up to 9 weeks.

Treatment

After the participants were asked to brush their teeth preceding the application, the complete dentition was isolated with cotton rolls and air-dried before EC40® was applied from a syringe with a blunt end needle in the fissures, approximal areas and to all tooth surfaces that were close to the gingiva. On average, approximately 1.2 ml varnish was applied containing 0.44 mg chlorhexidine in each application. Once EC40® tooth varnish had been applied, it was moistened to set and removed after 7.5 min according to the manufacturer's instructions. The application was repeated twice within 1 week. All varnishes were applied by the same operator.

Plaque sampling

Plaque samples were collected at baseline just before the first application, and at day 1, weeks 1, 2, 3, 6 and 9 after the third application. The participants refrained from oral hygiene 18 h before plaque sampling. They were not allowed to take any food or drinks for 2 h before sampling. At each visit, subjects rinsed with 10 ml of a 10% sucrose solution for 2 min to induce acid formation; 8 min thereafter a plaque sample was taken with a Teflon spoon from the buccal surfaces of the first and second molar in the upper left jaw. Plaque was immediately spun down at 16.100×*g* for 30 s into 50 μl MilliQ-water in a pre-cooled vial and put on ice until further processing within 1 h. The plaque sampling procedure itself never exceeded 1 min.

Plaque processing

The plaque samples were processed as described by Damen et al. [3]. In brief, the samples were heated to stop metabolic activity and cooled on ice to release the acids. Plaque samples were centrifuged in micro-spin vials (Ultrafree-MC 0.22 μm, Millipore, Bedford, MA, USA) and the plaque pellets stored at −80°C separately from their supernatants. Later, plaque pellets were thawed, resuspended in 200 μl MQ and sonicated on ice for 20×1 s (Kontes K-88140, Vineland, NJ, USA: maximum output). The protein content was determined [1] using the BioRad protein analysis kit with bovine serum albumin as standard (Sigma Chemical, St. Louis, MO, USA).

Organic acids analyses

In the supernatants, the organic acids were determined as their anions by capillary electrophoresis on the Waters Capillary Ion Analyzer (Milford, MA, USA). Sodium salts of formic, acetic, propionic, butyric, succinic and lactic acid (Sigma Chemical, St. Louis, MO, USA) were used to prepare mixture standard solutions in MilliQ-water. As an internal standard, 0.12 mM $NaNO_3$ was included in all samples. Calibration curves were made for each acid separately. Samples were analysed in duplicate and the Millennium Chromatography Manager Software version 3.05 was used for data analyses. Peak identification and peak area integration were manually corrected if necessary.

Statistics

The Statistical Package for the Social Sciences (SPSS version 10.0) was used to perform the statistical analyses. Paired-samples T tests compared the acetic and lactic concentrations at various evaluations to the baseline measurements. The minimally detectable difference be-

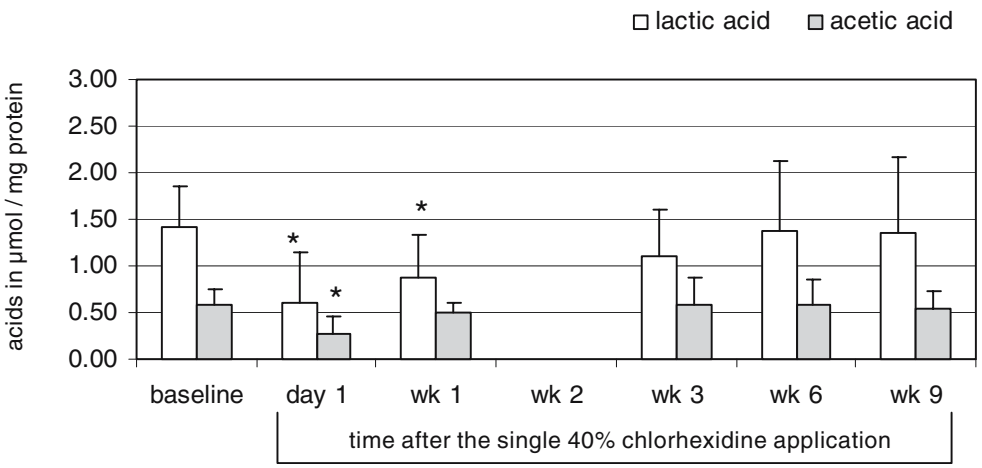

Fig. 1 Average lactic (*open square*) and acetic (*filled square*) acid concentrations (±SD) in µmol/mg protein in sucrose-challenged dental plaque after a single 40% chlorhexidine varnish application; $n=9$ (*significantly different from baseline $p<0.05$)

tween the response variables, using nine participants, the level of significance set at $p<0.05$ and a power of 0.8, is 0.30. This is based on the assumption that the within patient SD of the response variable is 0.2.

Results

All plaque samples were analysed for formic, butyric, propionic, succinic, acetic, and lactic acids. The formic, butyric, propionic and succinic acid concentrations were not included in the results because their combined contribution to the total acid concentration was less than 15%. We present the results of the single and triple applications in the same nine participants. The attrition of four students had not significantly affected the results of the single treatment as reported previously [8]. The results of the single chlorhexidine varnish application on the acetic and lactic acid concentrations in the buccal plaque of these nine subjects are shown in Fig. 1. After the single application, the concentration of acetic acid in sucrose-challenged plaque was significantly reduced at day 1 ($p=0.01$). The average post-sucrose lactic acid concentration decreased significantly from the baseline value of 1.42 (±0.44) µmol/mg protein to 0.60 (±0.54) at day 1 ($p=0.03$) and rose back to values of 1.10 (±0.51) at week 3 ($p>0.05$).

After the third application, plaque samples could not be taken on the first 7 days. Therefore, an additional examination was inserted after another week at which one of the subjects still produced too little plaque for sampling. The average post-sucrose acetic acid concentration did not differ from the baseline values 2 weeks after the third application (Fig. 2). The average post-sucrose lactic acid concentration at week 2 after the third application (1.22±0.53 µmol/mg protein) was lower ($p=0.05$) than the baseline value (1.64±0.69). At week 3 after the third application, the average lactic acid concentration (1.61±0.99) did not differ from the average baseline value. Despite the average reduction in lactic acid production 2 weeks after the third application, the effect had already disappeared in three subjects. In these three subjects, the effect had also disappeared very rapidly in our first study [8] (data not shown).

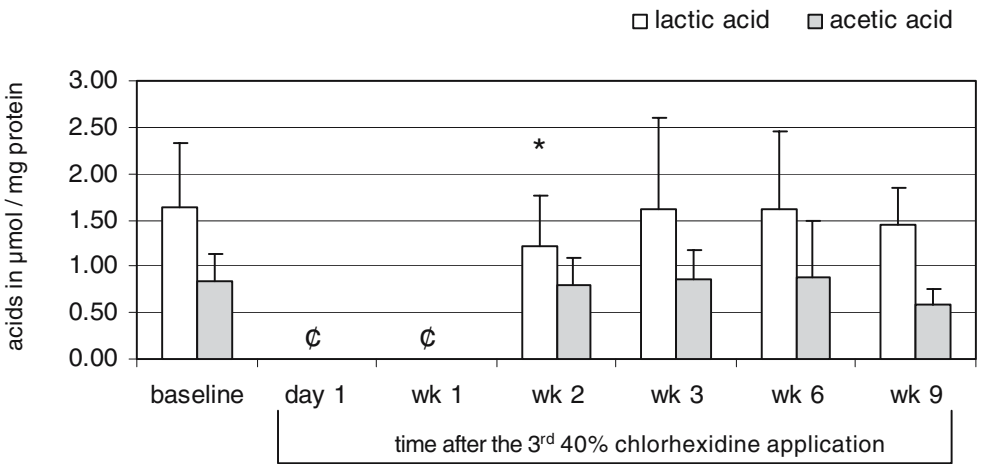

Fig. 2 Average lactic (*open square*) and acetic (*filled square*) acid concentrations (±SD) in µmol/mg protein in sucrose-challenged dental plaque after three applications with 40% chlorhexidine varnish; $n=9$ (*$p=0.05$; ¢=too little plaque to sample)

Discussion

Most studies on antimicrobials have measured both salivary and plaque mutans streptococci as an output parameter for the efficacy of antimicrobial treatments [6, 12].

In our single EC40® application study, we evaluated not only the number of salivary mutans streptococci but also the acidogenicity of dental plaque. We found that the reduction of salivary mutans streptococci was closely paralleled by the reduction of lactic acid in dental plaque [8]. But in some patients, salivary mutans streptococci recovered immediately while acid production continued to be reduced. Since acid is the metabolite of mutans streptococci causing caries, we chose acid production as the output parameter in the current clinical trial. In our opinion, the evaluation of the effect of our intensified antimicrobial treatment by acid concentrations in plaque is of sufficient clinical relevance.

Different modes of chlorhexidine application were explored in patients with moderate or high caries incidence [7, 14, 18]. Because the killing efficacy of chlorhexidine is supposed to increase with repeated applications [4], several authors have compared the effects of a single treatment on mutans streptococci in plaque to those of repeated treatments [9, 10, 17, 20]. Ie and Schaeken [10] reported a 2 months prolonged suppression of mutans streptococci in plaque in fissures of (pre-) molars after a double EC40® treatment compared to a single or a placebo treatment. In that study, however, the time-point of evaluating the double treatment was counted from the day of the first application. By doing so, they compared the 4-weeks results after the single application with the 3-weeks results after the double application. So, in fact, two variables—the treatment modality and the time since the last application—were evaluated, which could both explain the results. To avoid this complication, we chose to evaluate the intensified treatment on the described time-points after the third application. A comparison with the single application study evaluated purely the length of the effect of the treatment modality and not the benefit of the fact that the treatment itself took more time.

Others found that chlorhexidine had a continued suppressive effect on the number of salivary mutans streptococci after 4–7 applications of 1% chlorhexidine gel or 20% chlorhexidine varnish, respectively [11, 15]. Twetman [21] and Petersson [13, 21] studied the effect of a triple 1% chlorhexidine/thymol-containing varnish treatment (Cervitec®, Vivacare, Schaan, Liechtenstein) on the incidence and progression of approximal caries in schoolchildren anticipated to be at caries risk. Children who exhibited a less marked suppression of levels of interdental mutans streptococci after a triple 1% application showed a significantly higher progression than the children with a high suppression of mutans streptococci ($p<0.01$). Based on this literature, we also chose three applications within 1 week for our intensified 40% chlorhexidine varnish treatment.

Delayed bacterial regrowth after chlorhexidine varnish treatment is based on the principle of colonisation resistance [4, 17]. It was shown that the effectiveness of this mechanism depends on the percentage of the mutans population initially killed [5]. This may explain the benefit of the multiple treatments with lower concentrations (1–20%) of CHX [11, 13, 15, 21]: each treatment killed an additional fraction of the *S. mutans* population [16]. Then, after each treatment, it would be more difficult for the mutans population to recover. We had expected that repeated applications of 40% chlorhexidine varnish would also result in a longer period of reduced numbers of mutans streptococci and therefore of acid production in dental plaque. Although the total absence of dental plaque in the first week after the third application suggests a higher killing efficacy of the intensified program, this did not result in a prolonged reduction of the acidogenicity of dental plaque beyond a period of 3 weeks. Prolonged colonisation resistance is not necessarily achieved in dental plaque by an intensified chlorhexidine application regime. Probably, the killing of the mutans streptococci by the first 40% chlorhexidine application may have been so effective that a second and third application shortly after the first one did not reduce the remaining mutans population significantly further.

We studied the effects of a triple chlorhexidine application on acid production in dental plaque. From comparison with our previous study [8], we conclude that an intensive treatment with EC40® is not superior to a single treatment in reducing acidogenic properties of dental plaque, although it is more effective in inhibiting plaque regrowth during the first week after intensified treatment.

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