Effectiveness of Two Mouth Rinses Solutions in Arresting Caries Lesions: A Short-term Clinical Trial

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Purpose: To clinically evaluate the additional effect of adding 0.12% chlorhexidine digluconate (CHX) to a 0.05% sodium fluoride (NaF) mouth rinse in arresting active enamel caries lesions after 28 days.

Materials and Methods: A short-term double-blind clinical trial that included a total of 170 children, aged 11 to 15 years, with active smooth surface caries lesions (average 6.52) was conducted. The participants were enrolled and randomly distributed into two equal groups. Under the supervision of the research team, the children rinsed with a 15 ml solution of either 0.05% NaF (G1) or 0.05% NaF + 0.12% CHX (G2) for 1 min/day for 28 days. A clinical examination was carried out at the beginning and at the end of the study with children who underwent supervised tooth brushing. A calibrated examiner, who was unaware of the treatment given to each subject, examined all smooth surfaces dried with compressed air, isolated and illuminated with a reflector. The surface was considered active (A) or arrested (I). The frequency of A or I surfaces was evaluated by calculating the difference between the number found at the beginning and at the end of the trial.

Results: No significant differences were detected between the two groups with respect to caries lesion surfaces at baseline (6.49 ± 4.45 – G1, 6.55 ± 4.23 – G2, respectively), nor were differences found when age and gender were taken into consideration. Arrestment proportions were 84.4% (G1) and 85.3% (G2) (P = 0.71; not significant).

Conclusions: Although both solutions showed high arrestment proportions, the addition of CHX did not improve the arrestment capacity of the NaF mouth rinse.

Key words: chlorhexidine, dental caries, fluoride, randomised controlled trial

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Caries results from an imbalance between the phenomena of demineralisation and remineralisation. In situations of high cariogenic challenge, intra-oral pH is below 4.5 and the 'remineralisation' phase is inhibited (Thylstrup and Fejerskov, 1986). Under these conditions, the availability of more fluoride will not necessarily provide a better cariostatic effect (Ogaard et al, 1991). Several methods for enhancing the fluoride's cariostatic effect at low pH have been suggested, such as combining fluoride with antimicrobial agents (Büyükyilmaz et al, 1995). Chlorhexidine digluconate (CHX), a potent antimicrobial agent, inhibits the production of acid in dental plaque, reducing the drop in pH during cariogenic challenge (Rolla and Melsen, 1975). The pH of dental plaque quickly decreases after exposure to sugar and remains at the same low level for a varying period of time before slowly returning to its initial level. The pH of individuals with high caries activity has been shown to be lower (Stephan, 1944), in particular when dental plaque is associated with white spot lesions or deep dental caries lesions (Fejerskov et al, 1992).

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Clinical studies have reported a synergistic effect against *Streptococcus mutans* when a combination of fluoride and CHX is used (Luoma, 1972; Emilson et al, 1976). Because of its powerful bactericidal action against *S. mutans*, and its ability to reduce caries incidence (Zickert et al, 1983), CHX has been widely studied, especially in high-risk individuals (for meta-analysis, see van Rijkom et al, 1996). However, limited work has been carried out to identify the optimal combination of fluoride and CHX for the control of dental caries.

A Medline search was performed that was restricted to clinical trials, but with no language restriction, and using medical subject headings (MeSH) such as 'fluoride–chlorhexidine', 'chlorhexidine–fluoride', 'fluoride-combined chlorhexidine', 'chlorhexidine-combined fluoride', 'fluoride with chlorhexidine' and 'chlorhexidine with fluoride'. Twenty randomised clinical trials, published between 1966 and 2002, were identified. Eleven of these publications referred exclusively to the prevention of periodontal diseases, one investigated the adhesion strength of orthodontic brackets and the other eight papers referred to the prevention of dental caries (Keltjens et al, 1987; Keltjens et al, 1990; Spets-Happonen et al, 1991; Ullsfoss et al, 1994; Ogaard et al, 1997; Twetman and Petersson, 1997a; Twetman and Petersson, 1997b; Petersson et al, 1998). The selected studies analysed the effect of associating fluoride and CHX for the prevention of dental caries and either measured the decrease in caries or counted cariogenic microorganisms such as *S. mutans* and *Lactobacillus.* No data are available on the additional effect of using CHX for arresting active lesions, while concomitantly controlling the bacterial biofilm.

The purpose of this study was to assess the additional effect of adding 0.12% CHX to 0.05% NaF mouth rinse and clinically compare the capacity of solution of 0.05% NaF alone, to arrest active caries lesions on smooth enamel surfaces during 28 days of mouth rinsing in adolescents who did not receive other preventive interventions in the studied period.

MATERIALS AND METHODS

Subjects

The study was carried out in a public school in Florianópolis, Brazil. The study population consisted of pupils aged 11 to 15 years, enrolled at the school. Epidemiological study of dental caries, carried out using criteria established by the World Health Organization (WHO, 1997), was conducted in 2002 at the same school, involving all school children aged 11 and 12 years and the mean decayed, missing or filled teeth was found to be 1.4 (Bastos et al, 2004).

The research protocol was approved by The Ethical and Research Committee of Federal University of Santa Catarina (Protocol No. 154/2002), as well as by the relevant educational authorities, prior to study initiation. A letter was sent to the parents or guardians of the participants explaining the purpose of the study, its characteristics and its importance and requesting their agreement to the adolescent's participation and the parents were required to sign a consent form.

Eligibility criteria

Three hundred and sixteen adolescents had at least one active non-cavitated enamel caries lesion, on a free smooth enamel surface (buccal and lingual). However, 146 adolescents were excluded, due to the following exclusion criteria: adolescents with orthodontic brackets, dental prosthesis, prior aesthetic restoration of teeth and pregnant or breastfeeding girls. Twenty three adolescents were excluded due to refusal from his or her guardian to participate in the study. A total of 170 adolescents matched the inclusion criteria and took part in the study.

Study design

This was a double-blind, randomised clinical study for which the participants were randomised into two groups.

Sample size and power calculation

The sample size was calculated and detected a difference of 20% in the proportion of arrested lesions between the groups as follows: an alpha level of 0.05 (5%), test power of 80%, a proportion of 70% arrestment in those individuals exposed to supervised 0.05% fluoride mouth rinsing after 28 days (control group) and a proportion of 90% arrestment in the test group. A sample size of 71 individuals was estimated for each group, to which 15% more pupils were added to compensate for possible dropouts. Each group, therefore, enrolled 85

individuals. The Epi info™ software program (Georgia, USA) was used to calculate sample size (Dean et al, 1994).

Randomisation and blinding

A randomisation method was used to form the groups. A number was given to each eligible child and then put into a sealed opaque envelope. The envelopes were randomly selected by the supervisor (MLRJ) to allocate the individuals to each group of mouth rinsing solution, either 0.05% fluoride or 0.05% plus 0.12% CHX. Two pure solutions with the same pharmacological formulation were used, their only difference being the absence or presence of 0.12% CHX in solutions A and B, respectively. The solutions were placed in identical, amber-coloured, unlabelled containers, coded A and B. Neither the eligible children, the persons supervising daily mouth rinses, nor the examiner was aware of the contents of the solutions. The single examiner was also unaware as to the status of the participant as diagnosed at the previous examination and did not know to which group the adolescent had been allocated.

Mouth rinsing

The dental team instructed the teenagers to rinse with the 15 ml solution for 1 min every day during 14 consecutive school days, which actually represented a treatment period of 28 days. The other components common to the two solutions were 0.05% NaF, glycerine, non-cariogenic anise aroma, blue food colouring, preservative and vehicle q.s.

The solutions were specially prepared for the study and all had the same colour, flavour and artificial taste. According to the manufacturer (Formula & Ação, São Paulo, Brazil), the anise flavour successfully masks the bitter taste of CHX.

Personnel and training

The team consisted of a co-coordinator (MAP), two researchers and four dental students. The principal researcher (ARDG) clinically examined the caries lesions, the second (MLRJ) coordinated the tooth brushing and daily mouth rinsing supervised by the students, who transferred the participant's clinical data to their record cards. Brushing of the teeth was performed without the use of dentifrice, as Brazilian dentifrices contain sodium lauryl sulphate (SLS), which inactivates CHX, thereby requiring a minimum interval of 30 min between brushing the teeth and rinsing (Barkvoll et al, 1989), which would be an unreasonable interruption of the schoolchildren's routine. Although an effect of fluoridated water due to fluoride concentration of the drinking water and dentifrice in the arrestment of dental caries was expected, both groups were randomly exposed to these variables and any influence should, therefore, have been distributed randomly.

Calibration and intra- and inter-examiner reliability

Prior to the study, the principal researcher (ARDG) and reporter (RMF) were trained by the coordinator (MAP) to conduct calibration exercises using the methodology described by Peres et al (2001). A single calibrated examiner (ARDG) with high intra-examiner reliability conducted all the clinical examinations (kappa = 0.7). Another clinician (RSV), who did not participate in the data collection of this study, was the reference standard. Prior to the fieldwork, the examiner was instructed and calibrated to adopt dental caries lesion activity criteria of Nyvad et al for caries assessment in clinical trials (Nyvad et al, 1999). The examinations were performed in duplicate in 10% of the sample during the data collection to verify the intra-examiner agreement.

Clinical examinations and outcome

Two dental examinations were carried out; one at the beginning and one at the end of the study. Prior to the dental examinations, the pupils were asked to brush their teeth under a dental student's supervision, using a toothbrush provided by the research team, without dentifrice. The teeth were then dried with compressed air for 15 s, maintained by constant saliva aspiration and were illuminated using a standard dental light unit before being examined. If the examiner still found that insufficient plaque had been removed, the surfaces were gently cleaned using the side of the probe and gauze. The dental examinations were conducted on clean, dry and illuminated teeth using flat clinical mirrors in a room specifically reserved for this purpose. Each smooth surface was examined and a diagnosis was made according to the following criteria (Table 1) for non-cavitated caries previously described by Nyvad et al (1999) and Pinelli et al (2002).

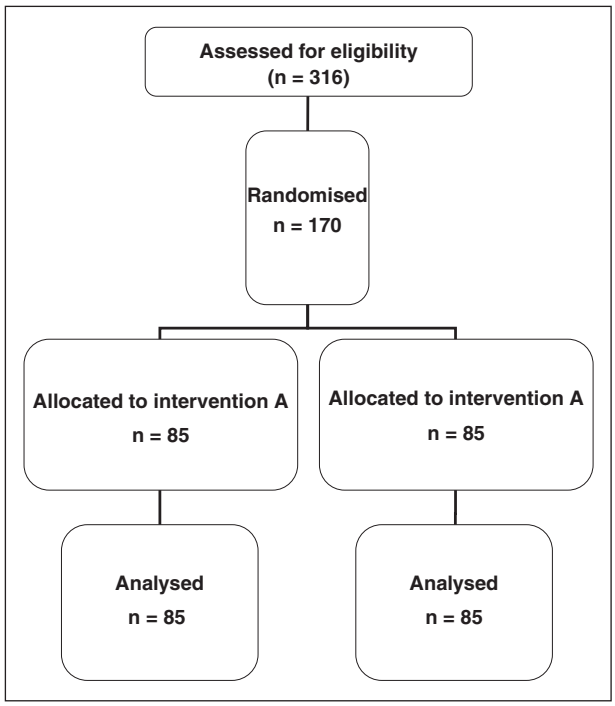

Fig 1 Progress through the different stages of the randomised control trial. A similar number of absentees were found in each group and were not considered dropouts. Based on the Consort statement flow diagram (Moher et al, 2001).

If the white spot was situated far from a healthy gingival margin, it was considered an arrested lesion (Pinelli et al, 2002; Maltz et al, 2003). These clinical diagnostic criteria have shown construct validation and predictive validity for the activity of caries lesions at the non-cavitated stage of diagnosis (Nyvad et al, 2003).

After satisfying all the inclusion criteria, the participant was then randomly allocated to the test or control group, as described above. The second examination followed the same criteria. Absenteeism was presumed when a teenager did not complete the total of scheduled mouth rinses or did not attend the final clinical examination. At the end of the study, those participants whose dental caries had not been arrested or who had other treatment needs were referred to the UFSC (Universidade Federal de Santa Catarina) or to the dental public health service.

Data handling and statistical analysis

Examiner reliability was measured using the kappa test on a surface basis. The Mann-Whitney *U* and chi-square tests were used to assess the differences in variables at the beginning of the study and the differences in arrested surfaces between the two groups after the trial. The mean, standard deviation and quartiles of active lesions at baseline and at the end of the study were calculated within the same group (G1 and G2). The difference between active lesions clinically identified at the baseline and at the end of the study was tested using the Wilcoxon matched pairs test. The SPSS software program (IL, USA) was used for the analysis.

Table 1 Criteria used to make diagnosis for non-cavitated caries

Code	Clinical condition	Clinical characteristics
(A)	Active caries lesion (non-cavitated)	Opaque, roughened and dull enamel surface
(I)	Inactive caries lesion (non-cavitated)	Smooth, shiny enamel surface with varying degrees of white or brown discolouration

RESULTS

One hundred and seventy participants were recruited to the study and equally distributed between the two groups. All participants remained in the study until its conclusion (Fig 1).

Table 2 confirms that possible confounding variables such as gender and age were evenly balanced between the study group (0.05% NaF with 0.12% CHX – G1) and the control group (0.05% NaF mouth rinse alone – G2). Moreover, the mean number of surfaces showing active lesions was not significantly different between the two groups at baseline (6.49 and 6.55, respectively).

Both groups (G1 and G2) showed a highly significant reduction in mean number of active lesions and in all quartiles of the frequency distribution during the study period (Tables 3 and 4).

Table 5 shows that after the period of treatment the arrestment proportions were high in both groups (84.4% and 85.3% for G1 and G2, respectively, $P = 0.71$; not significant). When the mean number of arrested surfaces was compared, G1 and G2 presented similar values (5.54 and 5.52, respectively). Subject absenteeism, which could have modified the results, was almost identical in the two groups.

DISCUSSION

Principal findings

After the end of the clinical trial, around 80% arrest of dental caries lesions was observed in both the

Table 2 Demographic and clinical characteristics of subjects in G1 (0.05% NaF mouth rinse) and G2 (0.05% NaF combined with 0.12% chlorhexidine mouth rinse) at baseline

Variables	G1 (n = 85)	G2 (n = 85)	*P*
% Female participants	58.8%	55.3%	0.64*
Age in years – mean (SD)	12.96 (1.38)	13.01 (1.34)	0.88**
Number of active lesions – mean (SD)	6.49 (4.45)	6.55 (4.23)	0.89**

SD: Standard deviation; *Chi-square test; **Mann-Whitney *U* test.

Table 3 Effectiveness of 0.05% NaF mouth rinse (G1) in arresting active caries lesions in enamel at the end of the study

	Mean	SD	Minimum	25th	Median	75th	Maximum
Active lesions at baseline	6.49*	4.45	1	3	5	9	21
Active lesions after the end of the study	0.95*	1.79	1	0	0	1	10

*$P < 0.001$ (Wilcoxon matched paired test).

Table 4 Effectiveness of 0.05% NaF combined with 0.12% CHX (G2) in arresting active caries lesions enamel at the end of the study

	Mean	SD	Minimum	25th	Median	75th	Maximum
Active lesions at baseline	6.55*	4.23	1	3	4	9	21
Active lesions at the end of the study	1.02*	1.60	0	0	0	1	8

*$P < 0.001$ (Wilcoxon matched pairs test).

Table 5 Effectiveness of G1 treatment (0.05% NaF mouth rinse) and G2 treatment (0.05% NaF combined with 0.12% chlorhexidine) in arresting caries lesions in active enamel subsurface at the end of the study

Variables	G1 (n = 85)	G2 (n = 85)	*P*
Number of active lesions – mean (SD)	0.95 (1.79)	1.02 (1.61)	0.34**
25th	0.00	0.00	
Median	0.00	0.00	
75th	1.00	1.00	
Arrested lesions/individual – mean (SD)	5.51 (3.86)	5.68 (4.07)	0.94**
25th	3.00	3.00	
Median	5.00	5.00	
75th	8.00	8.00	
Arrested lesions – proportion (SD)	85.4 (24.2)	85.6 (20.5)	0.66**
25th	73.03	75.00	
Median	100.00	100.00	
75th	100.00	100.00	
Subject absenteeism – mean (SD)	2.76 (2.34)	2.63 (2.76)	0.37**
25th	1.00	1.00	
Median	2.00	2.00	
75th	4.00	4.00	

SD: Standard deviation; **Mann-Whitney *U* test.

test and control groups. The mean number of arrested surfaces also presented similar values in both groups. Hence, the addition of CHX did not enhance the fluoride effect in arresting the activity of caries lesions.

No clinical studies have been identified that analyse transition proportions of caries lesions from the active to the inactive state using fluoridated combined with antimicrobial agents. This trial showed high arrestment proportions in both groups (Table 3), but did not detect any difference between the combined solutions and the fluoride alone, contrary to previous reports (Luoma et al, 1978; Spets-Happonen, 1991). The high proportions from active to inactive lesions are difficult to discuss due to the lack of these findings in the literature. What could be closer to these results were the high rates found in previous studies evaluating topical fluoride treatment in young teenagers (Baelum et al, 2003). High 'remineralisation' rates *in vitro* over a short evaluation time were also found following topical fluoride treatment: 1.8% per day during one month (Lagerweij and Ten Cate, 2002) and 5% per day for 9 days (Goorhuis and Purdell-Lewis, 1986), probably because the highest increase in remineralisation occurs in the first two weeks (Iijima et al, 1999).

High rates of arrested caries lesions were observed after a short length of time when topical fluoride was used: 1.8% per day during 1 month (Lagerweij and Ten Cate, 2002) and 5% per day during 9 days (Goorhuis and Purdell-Lewis, 1986). These findings suggest that clinical effects of topical fluoride in arresting caries lesions may occur in the first few weeks (Thylstrup et al, 1994; Iijima et al, 1999).

Strengths and weaknesses of the study

There is a greater prevalence of non-cavitated or pre-cavitated caries lesions compared with cavitated surfaces in economically developed countries (Ismail, 1997; Amarante et al, 1998; Ismail, 2004). Florianópolis has higher socioeconomic and health indicators than Brazil as a whole. In addition, it was found to have a low prevalence and severity of dental caries, mostly due to the fluoridated water supply and to the fluoridated dentifrices that are now distributed on a regular basis (Marcenes et al, 2001).

Fluoride sources, for example fluoridated dentifrice used at home and increased awareness of dental cleanliness as a result of enrolment onto the trial could certainly have contributed to the positive results achieved in this trial. In addition, it is important to observe that higher arrestment rates have been observed on free smooth tooth surfaces compared with other surfaces, potentially owing to abrasion from tooth brushing (Baelum et al, 2003). These facts may explain the high arrestment rates recorded in this study. The design, randomisation method, blinding and high intra- and inter-examiner reliability achieved in this study demonstrate its strong internal validity. Both rinsing solutions were identical to one another with respect to physical characteristics such as colour, weight, taste and smell, the only difference being the active pharmacological agent. They were specifically prepared for the study, but no commitment of any kind was given to the manufacturer to avoid conflict of interest.

The inherent subjectivity of the clinical examination can be avoided by using clear criteria-defining terms to measure the progression of the caries (Ismail, 2004). In addition to using clearly defined criteria, the double-blind nature of this study should have avoided detection bias. The calibration exercise adopted assured high intra-examiner reliability as found in this study and preceding studies that adopted the same procedures (Nyvad et al, 1999; Pinelli et al, 2002). Quantitative diagnostic methods were not used because such methods have shown low sensitivity and moderate specificity (Ismail, 1997; Bader et al, 2002) and until these emergent methods really prove superior to conventional diagnostic methods such as clinical examinations, they should not be recommended for clinical practice (Nyvad, 2004).

The dissimilarity of the studies published in the literature on the efficacy of CHX in the control of caries in terms of study design and outcome measures may have impaired the discussion of the present findings.

Ekstrand et al (2005) identified difficulties related to the examiner's visual and tactile assessment of the lesions activity. The intra-examiner agreement was only fair while the inter-examiner reliability was generally poor. The authors suggested that 3 to 4 weeks used in this pilot-study might be too short to evaluate changes criteria of lesions activity. Nevertheless, in provoked caries lesions, signs of regression were perceived after as little as 1 week (Holmen et al, 1987; Thylstrup et al, 1994), so it was expected that in natural circumstances, active lesions, visual and tactile changes might be detected after 3 to 4 weeks (Thylstrup et al, 1994). In this context, it is also important to consider a study that analysed the clinical behaviour of free smooth surfaces caries lesions monitored over 2 years, examining only

molar teeth from teenagers randomly allocated to three groups encouraged to use a 1,000, 2,000 or 2,500 ppm fluoride dentifrice, respectively (Neilson and Pitts, 1991). Although 74% of the lesions apparently remained static or regressed, the fluoride content of the dentifrices did not appear to affect the clinical appearance of the lesions.

Oral hygiene could be improved by including antimicrobial agents to control caries lesions activity in some specific situations. For example, when satisfactory oral hygiene is difficult to achieve or when there is dental caries activity in some specific surfaces, especially lingual surfaces, indicating risk of developed deep cavitations.

There are several possible explanations for the lack of difference between the two solutions in arresting active caries lesions. First, previous findings demonstrated the limited bactericidal effect, in which, 90 min following mouth rinsing, no further inhibition of its metabolic activity in dental plaque was detected (Giertsen and Scheie, 1995).

Studies with the objective of evaluating the ability of CHX to protect enamel against demineralisation may be more adequate for evaluating the effectiveness of CHX (Ten Cate and Marsh, 1994), in contrast to this present study that intended to evaluate the therapeutic effect of combined CHX-fluoride in arrestment of active caries lesions. This statement is based on the mechanism of action of CHX that consists primarily in interfering with bacterial metabolism and subsequently producing a secondary effect on the demineralisation of enamel. It was not the purpose of this study to evaluate inactive lesions or sound surfaces.

CHX did not add to the effectiveness of fluoride in controlling active caries lesions. In clinical situations, it is important to ensure simple treatment regimens to obtain compliance. Therefore, based on the present clinical trial we do not recommend the addition of CHX to NaF mouth rinses for the arrest of active caries.

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