Remineralisation of Carious Enamel Lesions after Application of a CHX/F-Mouthrinse Compared with Sole CHX- and Placebo-Application

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Purpose: The purpose of this cross-over, double-blind, placebo-controlled, randomised in situ study was to evaluate the remineralisation of demineralised enamel specimens using a CHX/NaF mouthrinse in comparison with a CHX- and a placebo-mouthrinse.

Materials and Methods: Twenty-four volunteers received intraoral appliances with mounted demineralised enamel specimens. They rinsed their mouths twice a day (10 ml for 1 minute) with the respective preparation. After 14 days, the in situ mineral gain and fluoride uptake were determined.

Results: Mineral gain was significantly higher after using CHX/F mouthrinse (358.4 ± 372.1 Vol.%·µm) compared with CHX (95.6 ± 192.2 Vol.%·µm) and placebo treatment (80.8 ± 159.6 Vol.%·µm). Moreover, CHX/F treatment (2751.1 ± 1494.9 µg/cm^3) resulted in a significantly higher fluoride uptake compared with CHX- (83.8 ± 94.0 µg/cm^3) and placebo- (136.6 ± 137.3 µg/cm^3) mouthrinses.

Conclusion: The in situ study approves that using a CHX/F mouthrinse supports the remineralisation of initial carious lesions.

Key words: caries, chlorhexidine, enamel, mouthrinse

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The mechanical reduction of plaque is today the basic standard in caries and periodontitis prevention. After oral surgery, during intermaxillary fixation or fixed orthodontic therapy, as well as for handicapped individuals, conventional oral hygiene is almost impossible and a chemical plaque reduction is necessary (Addy, 1986; Gjermo, 1974). Also, during the hygienic phase of periodontal therapy, chemical plaque control is recommended (Addy, 1986; Lang and Brecx, 1986).

Chlorhexidine (CHX) is a proven gingivitis-inhibiting agent (Mandel, 1988), powerful in plaque control (Addy, 1986) and the 'golden standard' in the prevention of plaque formation (Lang and Brecx, 1986; Quirynen et al, 2001).

The efficacy of CHX, used as an adjunct to the mechanical plaque control, has been positively reviewed (Axelsson and Lindhe, 1987). Its effectiveness in periodontal therapy (Bosman and Powell, 1977), during bimaxillary fixation (Krenkel and Rothler, 1979) and orthodontic treatment (Krenkel and Rothler, 1979) as well as for mentally retarded children (Bay and Russell, 1975) has been shown. However, the accumulation of plaque not only leads to gingival and periodontal reactions but also can cause carious demineralisation of

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the tooth's hard tissues (Loesche et al, 1984). Predominantly, different serotypes of *Streptococcus mutans* and *lactobacilli* are associated with the incidence of new carious lesions (Loesche et al, 1984). As a consequence of their metabolic activity, the equilibrium of hydroxyapatite and ions in solution is disturbed, thereby promoting further dissolution of hydroxyapatite (Ten Cate et al, 2003).

Therefore the outcome of caries prevention depends on two main factors, namely on the control of the cariogenic biofilm and on the prevention of demineralisation of the hard tissues. There is evidence that CHX application is efficacious in inhibiting growth of *S. mutans* and retarding acid production by cariogenic bacteria (Emilson, 1994; Giertsen and Scheie, 1995). It has been shown that CHX reduces the adhesion of *S. mutans* to the pellicle (Marsh, 1992) and influences the metabolism of the bacteria (Marsh et al, 1983).

Fluoride facilitates remineralisation of existing carious lesions and hampers demineralisation of enamel and dentin (Thylstrup, 1990; Rolla et al, 1993). It is also known that fluoride loses its therapeutic capacity under severe cariogenic conditions (Ullsfoss et al, 1994). Under these circumstances it is therefore suggested to remove, or at least disturb, cariogenic plaque in order to promote the caries preventive capacity of fluoride compounds (Ullsfoss et al, 1994). These observations suggest the development of a combined mouthrinse offering the antimicrobial and the hard tissue repairing principles together in one preparation.

A positive synergistic effect concerning plaque reduction and remineralisation of CHX and fluoride was discussed when the two substances were used intermittently (Laurisch, 1994). However, little is known about their efficiency while being used together in one solution. Jenkins et al (1993) previously conducted a clinical study to evaluate the antigingivitis efficacy of a combined CHX/F mouthrinse. They stated that the CHX/F mouthrinse could be used in those regimens considered for other CHX formulations and that the value of such a formulation in caries prevention would seem worthy of further investigation. Consequently, the aim of the present study was to evaluate the remineralising capacity of a CHX/NaF mouthrinse in situ.

MATERIALS AND METHODS

This three-way, cross-over, double-blind, placebo-controlled study was performed according to the guidelines of good clinical practice (GCP). The study was performed in full accordance with the Declaration of Helsinki. The study protocol, as well as all documents required for this study, were reviewed and approved by the University's independent ethics committee.

For determination of the number of participants in the study, we referred to the literature (Buchalla et al, 2002). According to these results regarding mineral gain and fluoride uptake, the statistician calculated including an estimation of dropouts.

The volunteers were residents of Freiburg, Germany, and surrounding villages with negligible tap water fluoride (badenova AG & Co., KG, 2005). The volunteers received a sequence number in the order of their appearance and the co-investigator informed them about the study orally and by an information sheet handed to every volunteer. Between the informing visit and signing of the informed consent, there were at least 24 hours for consideration. After signing the informed consent, demographic and ethnic data, medical history and concomitant medication were recorded. Changes that appeared during the study were logged in the case report form (CRF). The dentition and the oral mucosa were clinically examined. The volunteers were then screened according to the parameters shown in Table 1. For evaluation of the salivary flow rate and buffer capacity, CRT buffer kits (Ivoclar Vivadent, Ellwangen, Germany) were used. Plaque formation was evaluated by the plaque-formation rate index (PFRI) (Helm, 1986). Only volunteers fulfilling the inclusion criteria without violating the exclusion criteria were enrolled into the study.

Subsequently, impressions of the lower jaw were performed and intraoral appliances were fabricated using orthodontic wire (Remanium®; DENTAURUM JP Winkelstroeter KG, Ispringen, Germany) and resin (Orthocryl®; DENTAURUM JP Winkelstroeter KG).

Bovine incisors of the permanent dentition were used from animals that had been checked for BSE by an independent laboratory according to the German law. Three cylindrical specimens with a diameter of 4 mm were prepared from each of 360 incisors. One specimen was used for mineral analysis, one for fluoride analysis and one was kept as fluoride reference (Fig 1). All specimens were embedded in resin Technovit 4071® (Heraeus Kulzer GmbH & Co. KG, Hanau, Germany), ground and polished using wet sandpaper, merely removing the outer 100–200 µm. Then they were flattened from the dentin side. All 1080 specimens were kept in a demineralisation solution according to Buskes et al (1985), titrated to a pH of 5.00 and positioned on a shaking table. By shaking the containers and not actively rinsing the specimens, the specimens were prevented from erosion or mechanical damage. After six days, the specimens were plasma-

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Male or female between 18 and 70 years of age • Classified as healthy • At least 20 own teeth with average oral hygiene (tooth brushing at least twice a day) • Willing and able to give written informed consent before screening • Healthy or prosthetically, conservatively and periodontally restored dentition • Willing to abstain from fluoride-containing products and food (fluoridated tooth picks, fluoridated floss, black tea, green tea, fish) apart from the study products • Flow rate of stimulated saliva exceeds or reaches 0.7 ml/minute • Buffer capacity of saliva 'medium' or 'high' (CRT buffer kits)	• Ongoing dental treatment or any other medical treatment of the oral cavity • Any known allergy to previously used oral hygiene products and/or oral therapeutic agents and/or dental materials, which are used in the oral cavity or in the throat • Any known allergy to any of the ingredients of the study product or the standard toothpaste, which are used during the study and the wash-out periods • Current periodontitis or non-physiological tooth mobility • Any pathological change of the oral mucosa or gingivae • Heavy plaque formation • Eating disorders (bulimia, anorexia) • Use of prohibited treatments/therapies • Pregnant or breastfeeding • Participation in a clinical study within the previous 30 days

Table 2 Ingredients of the mouthrinses (active ingredients in bold type)

CHX/F	CHX	Placebo
• **0.2% Chlorhexidine digluconate** • **0.055 Sodium fluoride** • Water • Sorbitol • Glycerol • Cremophor RH 40 • Flavour • Citric acid • Dye	• **0.2% Chlorhexidine-digluconate** • Water • Propylenglycol • Glycerol • Cremophor RH 40 • Flavour • Citric acid • Dye	• Water • Propylenglycol • Glycerol • Cremophor RH 40 • Flavour • Citric acid • Dye

sterilised and mounted in the oral appliances facing to the cheek. Half of each specimen's surface, designated for the microradiographic analysis, was covered with Heliobond (Ivoclar Vivadent) before the insertion. During the whole study, the volunteers had to brush their teeth with non-fluoridated toothpaste (Aronal®, Gebro Pharma GmbH, Fieberbrunn, Austria). Before the volunteers received their appliances and between the study-periods, there were wash-out phases. Each lasted 7 days to minimise free fluoride in oral structures and thus to avoid a sequence effect.

The volunteers had to wear the appliances for least 21 hours. During meals, the subjects were allowed to take the appliances out and to store them in plastic containers on moist gauze. To ensure a certain comfort and to keep up the compliance of the subjects, they were allowed to brush the plastic surfaces of the appliance twice a day with a separate toothbrush and water. Toothpaste was not allowed. To simulate the absence of oral hygiene and to let plaque grow, the specimens were not cleaned. In order to log the times the appliances were not worn, a diary was used.

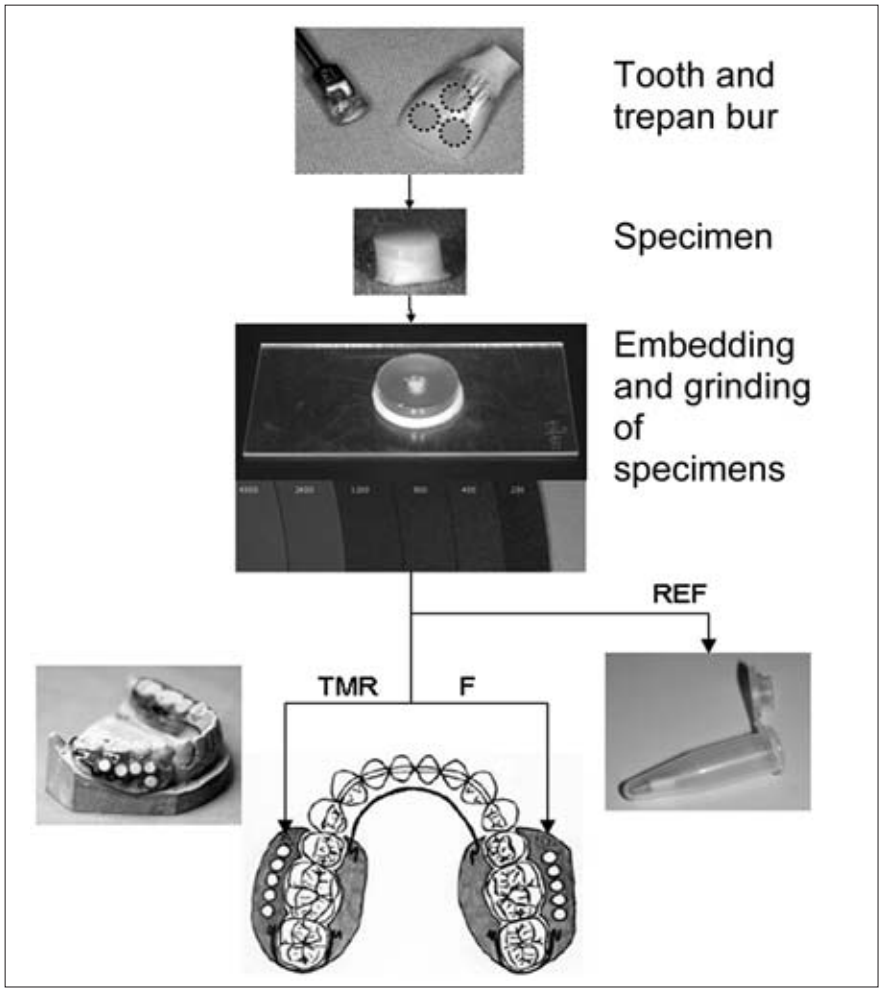

Fig 1 Processing of the specimens and appliance design. TMR, specimen for transversal microradiography; F, specimen intended for fluoride analysis; REF, reference specimen (basic fluoride level).

At the start of each of the three periods, the volunteers received their appliances as well as one of the three mouthrinses (Table 2). The treatment sequence was computer generated for every volunteer according to his or her sequence number. A statistician, who also did the later statistical analysis, generated the randomisation list. All study personnel were blinded during the study. The study product was delivered in identical containers. All three study products had the same characteristics such as colour, smell and flow behaviour. In case of an emergency, a treatment sequences list was kept in a sealed envelope at the study centre.

The volunteers rinsed their mouths twice a day for one minute with 10 ml of the respective mouthrinse. After 7 days, the subject came into the office for a check-up visit. Specimens were evaluated according to the bonding layer and medical and oral tissue status was examined. After 14 days, appliances were taken back, and specimens were stored in 'Eppendorf' cups. During the following wash-out period, the appliances were equipped with new specimens and treated as mentioned above (Fig 2).

As a main criterion, we determined if the use of the combination of CHX and NaF resulted in a higher mineral gain compared with a sole CHX treatment or a placebo treatment. Furthermore, we tested if there was a significant increase in remineralisation by using a CHX mouthrinse compared with the placebo treatment. As further study targets, fluoride uptake and change of the lesion depth were determined after the use of the CHX/F formulation and compared with the CHX and placebo treatment.

For mineral analysis, specimens were again embedded in resin and sectioned to a thickness of 100 µm ± 10 µm. For X-rays, a Cu Kα source at 20 kV and

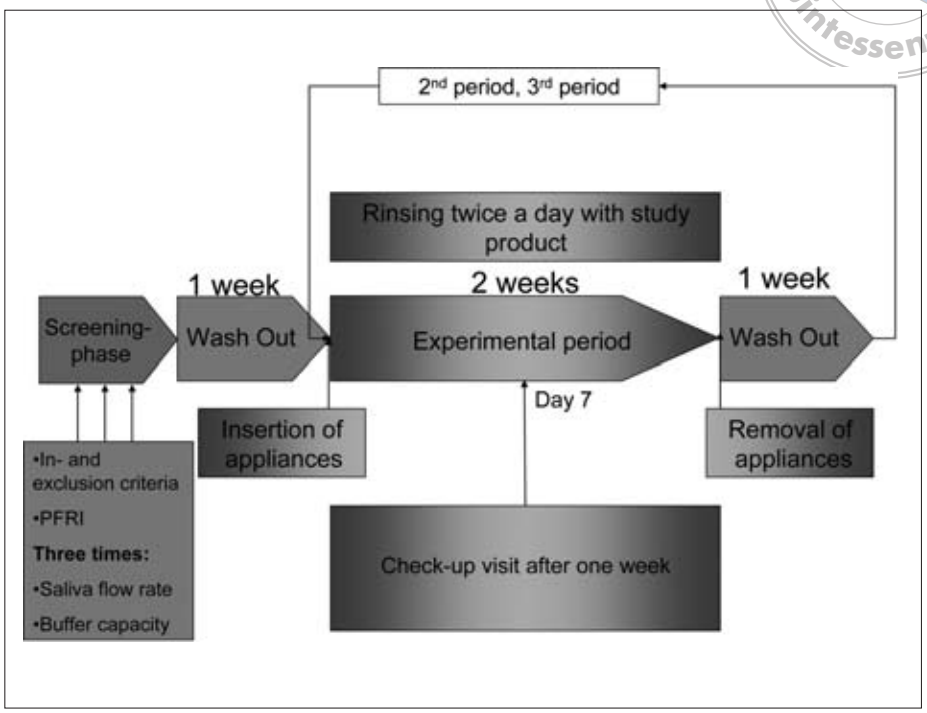

Fig 2 Schedule of the study design.

20 mA with an exposure time of 12 seconds was used. The microradiograph included an aluminium calibration step-wedge, and the slices were X-rayed perpendicular to the exposed and bonding-covered surface.

The microradiographic films (SO 253, Kodak AG, Stuttgart, Germany) were analysed using a stereo microscope (Axioplan, Zeiss, Oberkochem, Germany) with a mounted CCD-Camera (XC-77CE, Sony, Japan). For calculation of the mineral content and the lesion depth, TMR 1.25e (Inspektor Research Systems B.V., Amsterdam, The Netherlands) was used. Sound enamel mineral content was considered 87% by volume. Lesion depth was defined as the distance from the specimen surface to where 95% of sound mineral content was reached (Angmar et al, 1963). Correspondingly, the mineral change $\Delta\Delta Z$ (Vol. % • µm) during the treatment was calculated, by subtracting ΔZ_{test} (exposed) from ΔZ_{ref} (reference/bonding covered) ($\Delta\Delta Z = \Delta Z_{ref} - \Delta Z_{test}$). Positive results were considered as remineralisation and negative results as demineralisation. The same procedure was performed to analyse the lesion depth. The difference between the lesion depth of the reference area and the lesion depth of the exposed area ($\Delta l = l_{ref} - l_{test}$) was calculated. Positive results were considered as remineralisation, negative results as demineralisation.

For fluoride analysis, 100 µm of the reference and the exposed specimen was ground off and the enamel was dissolved in 0.5 ml of 0.5 mol/l $HClO_3$ and buffered with 2.5 ml of total ionic strength adjustment buffer II (TISAB II). Then the fluoride concentration of the solution was measured using an ion-selective electrode (Thermo Electron Corporation, Environmental Instruments Water Analysis, 166 Cummings Center Beverly, MA 01915, USA). Total fluoride content was calculated in $\mu g/cm^3$. The fluoride contents of the reference samples, F_r, were subtracted from those of the tested samples, F_t, ($\Delta F = F_t - F_r$). A positive ΔF was interpreted as a fluoride uptake, a negative result as fluoride loss.

Statistical analysis

For confirmative statistical testing an analysis of variance (ANOVA) model was used, including the treatment sequences as 'between subjects' factors' and the treatment conditions as 'within subjects factors'. In the case of significant results of the ANOVA, intergroup comparisons were made by paired t-tests. Adjustment of type I error due to multiple testing was not necessary due to *a priori* ordering of the hypotheses. Hypothesis testing had to be stopped if a null-hypothesis could not be rejected on the 5% error level. This procedure maintained a constant global level of 5%.

RESULTS

Demographic results

Nineteen out of 24 study subjects were female (79.2%). The average age of all participants in this study was 35.1 (± 11.3) years. The age ranged from 21 to 60 years. All subjects were Caucasians and written informed consent was obtained from all of them. All subjects fulfilled the inclusion criteria at the beginning of the study. Exclusion criteria were not applicable in any of the cases. The mean DMFT index was 9.9 and the PFRI scores ranged from I to III. The status of the oral soft tissue was rated 'normal' in all cases. The mean salivary flow rate amounted to 1.7 ml/minute at the screening visit. The buffer capacity of the saliva was classified 'medium' (8 subjects) or 'high' (16 subjects). Thus all subjects screened were qualified for enrolment into the study. Of 24 volunteers, 23 finished the complete study, but only 21 were finally analysed. Two volunteers did not use the adequate amount of mouthrinse (which was ± 25% of the calculated amount). One volunteer aborted the third period one day earlier due to family affairs.

Efficacy results

Mineral change

After 14 days, a significantly higher mineral gain could be observed in the CHX/F group compared with the CHX and the placebo groups. Mineral gain amounted to 358.4 ± 372.1 Vol.% • µm in the CHX/F group compared with 95.6 ± 192.2 Vol.% • µm in the CHX group ($p = 0.001$) and 80.8 ± 159.6 in the placebo group ($p = 0.005$). Mineral gain after using CHX and placebo mouthrinse was not significantly different ($p = 0.803$) (Fig 3A).

Fluoride uptake

The same ranking could be observed regarding fluoride uptake. Application of CHX/F resulted in a significantly higher fluoride uptake (2751.1 ± 1494.9 µg/cm^3) compared with CHX treatment and placebo treatment ($p < 0.0001$). Mean values of the CHX and placebo groups indicated that the fluoride concentrations were below the detection level of the used fluoride electrode in these groups and for this reason the values are not given (Fig 3B).

Lesion depth

Treatment with the CHX/F mouthrinse resulted in a statistically significant higher reduction of lesion depth (5.8 ± 6.0 µm) compared with the placebo treatment (1.8 µm ± 5.5, $p = 0.037$). However, no significant reduction was found after the application of the CHX mouthrinse (2.6 ± 7.8 µm, $p = 0.069$) compared with CHX/F and no significant differences between CHX and placebo ($p = 0.729$) (Fig 3C).

Safety evaluation

Twenty-four study subjects (100%) suffered from one or more adverse events (AEs) during the study periods. In total, 63 adverse events were documented. Of these, 29 AEs occurred under CHX/F treatment, 21 AEs under CHX treatment and 12 AEs under placebo treatment. One AE was observed during the wash-out period (after the placebo treatment).

One AE, which occurred under CHX/F treatment ('bad taste'), was rated to be in a definite relationship with the study medication. For the other AEs, only a probable relationship to the study medication could be established.

The most frequent types of AE were symptoms that occurred in the oral cavity. Dysgeusia, burning of the oral mucosa, changes in viscosity of the saliva and changes of the colour of the teeth have been classified under these oral cavity symptoms. The second most frequent type of AE was headache.

DISCUSSION

One aim of this study was to evaluate if fluoride, in a CHX solution, is able to serve as a remineralising catalyst and is superior to the sole remineralisation effect of saliva in the presence of CHX. It was shown that remineralisation after CHX/F treatment is superior compared to CHX or placebo treatment. This result was statistically significant on a global level of alpha = 5%. Thus it can be concluded that a CHX/F mouthrinse might play a valuable role regarding caries prevention. Interestingly, not only mineral gain was increased significantly in the CHX/F group but also lesion depth was reduced reasonably. From in vitro and in situ studies, it is well known that progression of carious lesions can be stopped or even reversed under the influence of fluoride (Thylstrup, 1990). However, many of those studies were performed using clean tooth substrate. In the present study, the enamel specimens were recessed

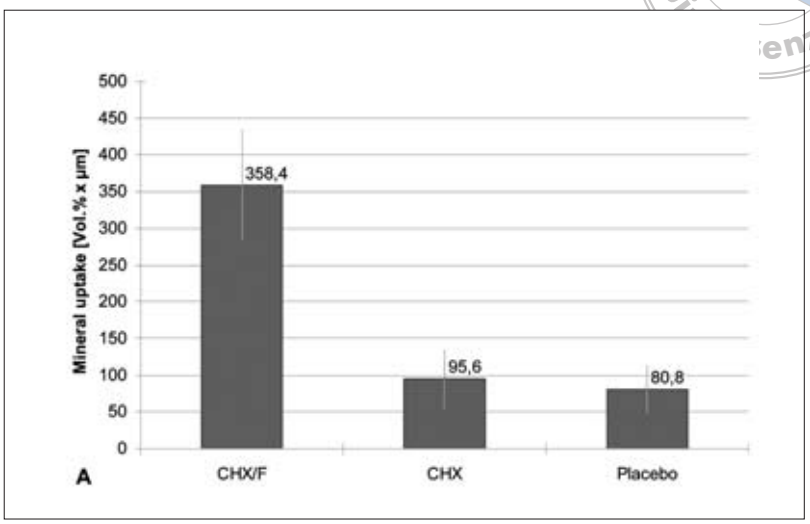

Fig 3a

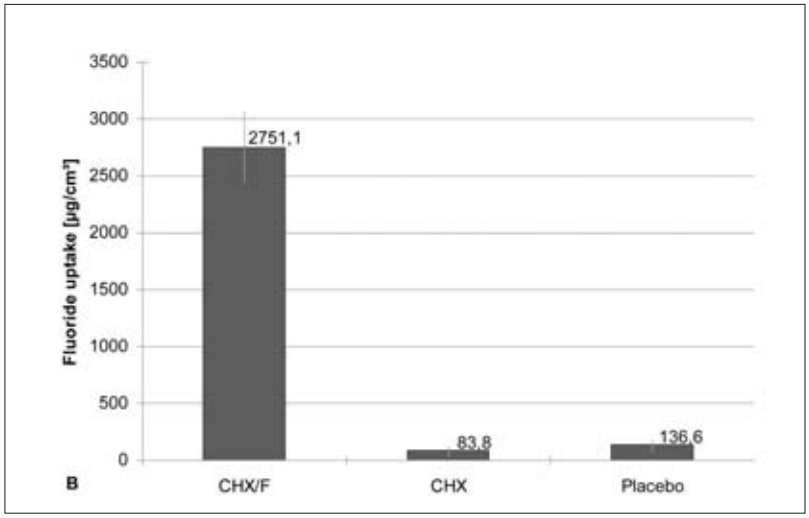

Fig 3b

Fig 3c

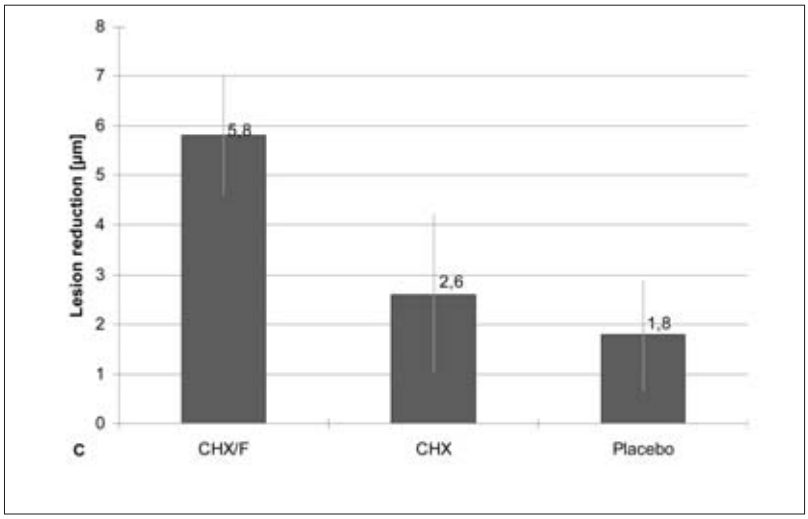

Fig 3a to c Means and standard errors of the means (represented by error bars) for (A) mineral uptake, (B) fluoride uptake, and (C) reduction of lesion depth after the use of the different mouthrinses for 14 days.

and consequently plaque was allowed to grow on them. Since CHX might act antibacterially while reducing plaque acidogenicity (Ullsfoss et al, 1994) fluoride was able to display its remineralising capacity despite the fact that plaque was present. In this context (Ullsfoss et al, 1994), the present results could also demonstrate that the use of CHX/F mouthrinse hampers enamel mineral loss (demineralisation) almost entirely even under severe cariogenic conditions. Moreover, the caries-protective efficacy of the combined preparation was superior to what has been observed with F-rinse alone.

The superior behaviour of CHX/F mouthrinses may be explained partly by the fact that the presence of fluoride strongly enhances the adsorption of CHX to hydroxyapatite and enamel (Ben-Yaakov et al, 1984) and that co-administration of F and CHX shows a higher diffusion rate in enamel for each compound than when separately diffused (Linden et al, 1986). This may also be the explanation for the high fluoride uptake of demineralised enamel in the present study.

Although a statistically significant better global remineralising efficacy was observed, this effect was not fully independent of the sequences in which the treatment had been administered. The highest mineral gain was observed in the CHX/F group when the sequence was CHX – placebo – CHX/F, followed by the sequence placebo – CHX/F – CHX, and CHX/F – CHX – placebo respectively. It might be speculated that the volunteers were adapted to the strange taste of the mouthrinses and rinsed more intensively when the CHX/F mouthrinse was used in the second and third experimental periods. Consequently, the enamel specimens were moistened more extensively with the active ingredients. A further aspect of this sequence effect might be that the previous treatment with CHX probably suppressed re-growth of plaque.

Several studies could be interpreted to show that there is only a negligible negative side effect of fluoride on the plaque-inhibiting efficacy of CHX. Nuuja et al (1992) found similar plaque weights and periodontal indices for a combination of fluoride, CHX and Xylitol compared with CHX. Furthermore, Luoma (1992) found a reduction in gingival bleeding and a reduction in caries increment when a combination of fluoride and CHX was used over 2 years.

Summarising the results of the present study, it can be concluded that fluoride exerts a reasonable remineralising capacity even when combined with CHX in the same mouthrinse. Referring to the results of Ullsfoss et al (1994), it can be speculated that the combined application of CHX and fluoride exerts a better cariostatic efficacy than fluoride alone. This is in agreement with studies conducted by Luoma (1992). The combined CHX/F mouthrinse might be a useful adjunct to the usual oral hygiene measures, particularly for patients with a high risk of caries, e.g. disabled persons, after periodontal surgery procedures, dental traumas or bimaxillary fixation. No serious AEs and no severe adverse drug reactions were observed. However, rinsing with the mouthwashes, especially with CHX/F and CHX mouthrinses, was frequently associated with dysgeusia and irritations of the oral mucosa, gingiva or tongue. Due to these reported AEs (side effects), its use should be limited in order not to tire patients' compliance.

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