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Effect of stannous fluoride toothpaste on erosion-like lesions: an *in vivo* study

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It has recently been shown that stannous fluoride (SnF_2), in the form of aqueous solutions and as toothpaste, can reduce the dissolution of enamel in erosive acids *in vitro* and *in situ*. The aim of this study was to compare the effect of toothpastes containing SnF_2 or NaF on enamel dissolution using an *in vivo* model. Four healthy anterior teeth in each subject ($n = 20$) were exposed to diluted citric acid (100 mmol l^{-1} or 10 mmol l^{-1}) applied using a peristaltic pump (5 ml @7 ml min^{-1}) and the acid was collected in a test tube before and after application of the respective toothpastes (etch I and etch II). Toothpaste was applied to the labial surfaces with a soft brush (four applications, each of 1-min duration), with gentle water rinsing between applications. Each subject had one pair of teeth treated with each of the test toothpastes. Enamel dissolution was examined by assessment of calcium content in the citric acid applied before and after the treatment with toothpaste. The results indicate that the SnF_2 toothpaste markedly reduced the dissolution of teeth *in vivo* (etch II < etch I), whereas the NaF toothpaste provided no protection (etch II > etch I). Toothpaste appears to be an acceptable vehicle for SnF_2 and maintains the dissolution-reducing effect exhibited by aqueous solutions of this fluoride salt.

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Dental erosion is a clinical problem in many countries (1, 2). Whereas knowledge concerning the etiology of dental erosion is widespread, no generally accepted preventive methods are available other than, for example, changing dietary habits when consumption of excessive amounts of carbonated beverages and fruit juices is the main causative factor (3). Fluoride has been studied as a preventive treatment for dental erosion for many decades, and a recent review of this topic concluded that prolonged applications of highly concentrated fluoride agents have a positive preventive effect (4). Although the clinical application of stannous fluoride (SnF_2) was suggested as a treatment method against tooth erosion as long ago as the late 1960s (5), more recent reports have supported these earlier findings (6–8). The dissolution-reducing effect of SnF_2 on enamel in the pH region related to dental caries is well established (9), whereas the corresponding effect against dental erosion has recently been demonstrated *in vitro* using an aqueous solution (6). The reduced dissolution of enamel exposed to acids appears to be associated with the formation of a protective surface layer (10). Toothpastes containing SnF_2 have been extensively used in the past and are known to provide protection against caries (11). Recent *in situ* studies indicate that toothpastes containing SnF_2 also protect against erosive acids (8).

The aim of the present *in vivo* study was to examine whether a SnF_2-containing toothpaste can protect natural dental enamel in healthy subjects teeth from the effects of dilute citric acid and thus be a possible prophylactic agent against dental erosions. A sodium fluoride-containing toothpaste was included as a control. The hypothesis was that SnF_2-toothpaste would protect the enamel against erosive acids.

Material and methods

Test subjects

Twenty subjects (age range 19–50 yr, mean age 31 yr, median age 27 yr) were recruited to take part in the study. Only those in good general health and with healthy maxillary anterior teeth were included in the study. Subjects were excluded if they had used SnF_2-containing toothpaste in the weeks leading up to the experiment, or if their teeth already showed obvious signs of erosive damage. The subjects were given written information about the study and informed consent was obtained prior to commencing the experiment. The protocol was approved by The National Committees for Research Ethics in Norway (REK-Sør: S-05223).

Clinical procedure

The clinical procedure was carried out in a dental surgery with the patient sitting upright in the dental chair. Without any cleaning, teeth 11, 12, 21 and 22 were isolated using cotton wool rolls and separated with plastic strips (Odus Universal Strips; Odus Dental, Zurich, Switzerland) and a light-bodied impression material (3M ESPE Permadyne

Fig. 1. Clinical photograph showing isolation of the teeth in one of the test subjects using light-bodied impression material (3M ESPE Permadyne Light Body).

Light Body; 3MEspe, Seefeld, Germany) was injected on to the palatinal surfaces to prevent exposure of these surfaces to citric acid (Fig. 1). The teeth were treated in pairs, whereby in most cases teeth 22 and 11 were treated at the same time followed by teeth 21 and 12. Using a peristaltic pump (flow: 7 ml min^{-1}; LKB 12000; Variopex, Bromma, Sweden), 5 ml of diluted citric acid (Sigma-Aldrich, Steinheim, Germany), either 100 mmol l^{-1} (pH 2.2) or 10 mmol l^{-1} (pH 2.7), was dripped on the labial surface of each tooth and the acid from the pair of teeth was collected together using a funnel attached to a test tube (etch I) (Fig. 2). The teeth were then rinsed with water using a triple syringe and the rinse water was collected in a plastic cup for disposal. The pairs of teeth were then treated with one of the two fluoride toothpastes. Following fluoride treatment, the teeth pairs were then re-exposed to 5 ml of citric acid and the acid collected in the same way as described above (etch II, Figs 1 and 2). Etch I and II acid samples were coded and stored cold until calcium analyses were carried out blind.

Fluoride toothpastes

Teeth 11 and 22 were treated with commercially available sodium fluoride toothpaste (NaF, 0.15% F^-) which was carefully applied with a small brush. After 1 min the teeth were rinsed with water using a triple syringe and dried gently before the procedure was repeated. The rinse water

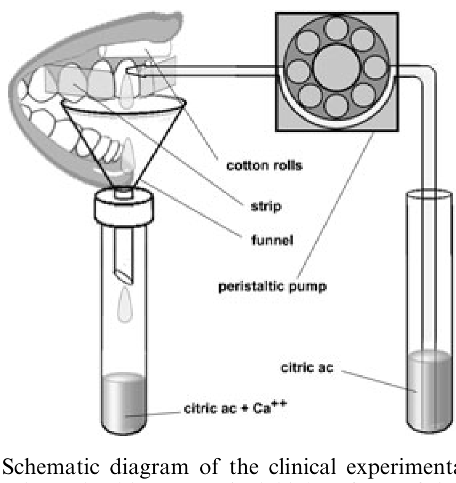

Fig. 2. Schematic diagram of the clinical experimental set-up showing the peristaltic pump, the labial surfaces of the teeth to be tested, and the funnel with test tube to collect the citric acid (citric ac) etch solution.

was collected using a plastic cup. The toothpaste was applied four times, for 1 min each application, and rinsed with water between applications. Teeth 21 and 12 were then treated four times with commercially available SnF_2 toothpaste (0.4% SnF_2, 1.0% stannous pyrophosphate: 0.10% F^-), as described for the NaF toothpaste. Following the last toothpaste applications the teeth were rinsed very thoroughly and gently dried. The test subjects rested in the chair for 1 min prior to etch II.

Calcium analysis

Enamel dissolution of the individual teeth was assessed by measuring the calcium content of citric acid from etch I and etch II. A Model 3300 Atomic Absorption Spectrometer (Perkin Elmer Analytical Instruments, Norwalk, CT, USA) was used for the analyses, which were performed blind.

Statistics

The difference in the amount of calcium released from the enamel of the teeth before and after fluoride treatment was taken to be caused by the respective fluoride treatments. The average calcium concentrations (in p.p.m.) and the percentage of control levels were calculated (etch II compared with etch I) for each treatment group, and a paired *t*-test was used to compare each fluoride treatment for both acid concentrations. The statistical package SPSS 11.0 for Windows (SPSS Inc, Chicago, IL, USA) was used for all analyses.

Results

The results are shown in Table 1. When applying the 10 mmol l^{-1} citric acid etch, the SnF_2-containing toothpaste provided significant protection against calcium loss compared with the NaF toothpaste. The reduction of dissolution was 20% when compared with the etch I control (Table 1). The NaF-containing toothpaste provided no dissolution reduction against the citric acid etch and a further loss of calcium was observed. When 100 mmol l^{-1} citric acid was used, neither the NaF- nor the SnF_2-toothpaste provided any significant reduction of dissolution of the enamel surfaces (Table 1). However, following treatment with NaF toothpaste, a marked increase in enamel dissolution was observed.

Discussion

Dental erosion is becoming an increasingly important clinical problem, and the treatment of serious cases is often both difficult and expensive. Although there are many complicating factors, there is a great need for more clinically relevant studies that examine possible preventive treatment measures for this problem. The present *in vivo* model was developed in order to go one step further towards a full clinical study but still allow validation of the method with respect to the results of preliminary *in vitro* studies. The effect of acid on the dissolution of natural dental enamel in the oral cavity, and treatment of the resulting erosion-like lesions with

Table 1

Results of the clinical experiment on 10 test subjects where the etchant was 10 mmol l^{-1} citric acid or 100 mmol l^{-1} citric acid, showing the change in enamel dissolution expressed as a percentage of the control, standard error values and P-values

	SnF_2 toothpaste			NaF toothpaste		
Citric acid concentration	Etch I (control) (p.p.m. Ca)	Etch II (p.p.m. Ca)	Percentage of control	Etch I (control) (p.p.m. Ca)	Etch II (p.p.m. Ca)	Percentage of control
10 mmol l^{-1}	0.816 ± 0.150 $P < 0.05$*	0.649 ± 0.126	79.27 ± 7.68	0.751 ± 0.137 $P < 0.001$*	1.502 ± 0.188	200.00 ± 36.840
100 mmol l^{-1}	3.62 ± 0.467 $P = 0.367$*	3.43 ± 0.355	94.96 ± 5.350	2.80 ± 0.276 $P < 0.001$*	6.42 ± 0.468	229.43 ± 25.79

Data are expressed as mean values and standard error, based on 10 test subjects.
*The *P*-values were calculated from paired *t*-tests comparing etch I and etch II.
p.p.m., parts per million; SnF_2, stannous fluoride.

fluoride in the form of toothpaste, was investigated. This model will be developed further in order to simulate conditions that are even more similar to clinical reality. Calcium analysis has recently been evaluated to correlate well with other methods in the quantitative assessment of erosive demineralization (12). Following pilot studies, the use of impression material was found to be essential in order to inhibit the transport of fluid to the lingual aspects of the teeth, giving a large variation in results. In this way only the labial surfaces were exposed to the citric acid.

In the present *in vivo* study, using teeth in subjects under controlled test conditions and using a citric acid concentration of 10 mmol l^{-1}, a 20% dissolution reduction of tooth enamel was demonstrated following treatment with the conventional SnF_2-containing toothpaste. In addition, an apparent remineralization seemed to have occurred, probably as a result of the Sn and fluoride-containing layer formed. This represented a considerable preventive effect when compared with the lack of protection provided by treatment with the conventional sodium fluoride-containing toothpaste. The lack of effect by the NaF toothpaste was not surprising; however, the magnitude of the difference was unexpected. Remineralization is the essential mechanism behind sodium fluoride treatment, involving fluorapatite deposition, a reaction that does not operate at pH levels below 4.5 (13). Previous studies that have reported good effects with sodium fluoride have involved very high concentrations of this fluoride salt (14). The reduced dissolution experienced after treatment by SnF_2 observed in the present study may have been affected by the demineralized state of the pre-etched enamel (following etch I). Pilot *in vitro* studies carried out prior to the present *in vivo* study, which did not include pre-etching, did not show such a good dissolution-reducing effect, confirming the trend seen by Ganss and co-workers in their *in vitro* and *in situ* studies (7,8). Pre-etched enamel would provide abundant amounts of calcium and acidic phosphates that presumably provide substrate for the tin layer and the calcium fluoride-like material protecting the surface.

The experimental design involved individual tooth pretreatment with citric acid and measurement of calcium found in this acid (etch I). The teeth were then treated individually with toothpaste, followed by a second etch with citric acid (etch II). In this way each tooth functioned as its own control. The design was considered to mimic possible localized treatment of eroded teeth. Citric acid at pH 2.7 was chosen as a severe and relevant challenge as this acid is present in orange, lemon, and grapefruit juices. Pure citric acid was chosen because the most commonly used natural products also contain calcium and phosphate (3) which would interfere with the calcium analysis and reduce the erosiveness of the acid component. A stronger citric acid concentration at pH 2.2 was included to assess the potential of the SnF_2 toothpaste. The SnF_2 toothpaste provided some dissolution-reducing effect, whereas treatment with NaF toothpaste resulted in further excessive loss of calcium. The total difference between the two treatments was surprisingly as much as 221% for the 10 mmol l^{-1} citric acid, as mentioned above, and 234% for the 100 mmol l^{-1} citric acid.

Toothpaste application on the teeth lasted for 4 min and was divided into four separate 1-min applications, which is longer than the average time of a tooth brushing procedure. However, it can be argued that the toothpaste application on the teeth in this experiment may be less effective than application by tooth brushing in the normal situation. Normal tooth brushing allows greater contact of the toothpaste with the tooth surface as it mixes with the saliva in the oral cavity. On the other hand, the combination of toothpaste and physical movements of a toothbrush may result in more mineral loss. It should also be noted that the present study only estimated the effect of one application (or four short applications) on the teeth. It is possible that daily use over a period of weeks or months might give an enhanced effect; however, this needs confirmation. It is therefore planned to carry out further experiments using this model where, following etch I, the subjects will use the toothpaste over a period of time (under standardized conditions) before etch II is performed.

The experiments thus supported the hypothesis that SnF_2 toothpaste is able to protect enamel against a weak erosive acid. Furthermore, the results also support the concept that toothpaste may be a suitable vehicle for

SnF_2 designed to reduce the development of dental erosions. It is likely that the mechanism of erosion inhibition is similar to that for caries inhibition and is probably based on a combination of stannous ions and calcium fluoride; where stannous ions provide a layer of tin ions adsorbed to the tooth surface and deposition of calcium fluoride on (and presumably in) the tin layer. The tin layer alone provides no protection as stannous chloride gives a tin layer but has no clinical effect (15). SnF_2 is acidic (pH 3 and 4 in aqueous solution or in toothpaste, respectively). At this pH, remineralization, which is the main aspect of the cariostatic mechanism of NaF or monofluorophosphate (MFP), would not occur, as discussed above (13).

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