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Effect of Stannous Fluoride and Dilute Hydrofluoric Acid on Early Enamel Erosion over Time in vivo

C. Hjortsjö[a] G. Jonski[b] P.S. Thrane[c] E. Saxegaard[a] A. Young[d]

[a]Department of Prosthodontics, [b]Oral Research Laboratory, [c]Department of Periodontology and [d]Department of Cariology and Gerodontology, Faculty of Dentistry, University of Oslo, Oslo, Norway

Key Words
Dental erosion · Hydrofluoric acid · Stannous fluoride

Abstract
Recent experimental in vivo studies have shown that aqueous solutions of stannous fluoride (SnF_2) and hydrofluoric acid (HF) can reduce enamel solubility after 5 min. The aim of this study was to evaluate the longer-term protective effect of SnF_2 (0.78%, pH 2.9) and HF (0.2%, pH 2.0) (both ~0.1 mol/l F) using the same experimental model. Labial surfaces of healthy anterior teeth (all four surfaces when possible, otherwise a pair of surfaces) in 103 subjects (n = 399 teeth) were exposed to citric acid (0.01 mol/l, pH 2.7). The acid was applied using a peristaltic pump (5 ml, 6 ml/min) and was collected in coded test tubes (etch I). The test solutions were then applied to the same surfaces of the teeth (1 min, 6 ml/min). After either 1, 7, 14 or 28 days, citric acid was again applied to the same surfaces and subsequently collected (etch II). Enamel solubility was examined by assessment of calcium concentration in etch I and etch II solutions using atom absorption spectroscopy. Median values were calculated for all time periods and statistical analysis was carried out using the Wilcoxon signed-ranks test. Results showed that HF reduced enamel solubility by 54 and 36% after 1 and 7 days, respectively. After 14 and 28 days, there was no longer any effect. SnF_2 showed no protective effect after the first day. Given these results, repeated application of HF and especially SnF_2 may be necessary to improve the protective effect of these fluorides, and this requires further testing.

Frequent exposure to intrinsic and extrinsic acids can potentially cause dental erosion [Scheutzel, 1996; Zero, 1996]. The pathogenesis of erosion is complex, with many possible contributing factors, most notably the frequent intake of acidic drinks or food [Bartlett, 2005; Linnett and Seow, 2001; Lussi et al., 2004]. Acid exposure from an acidic diet comes directly from the consumed product and will only be effective in the cavity for a couple of minutes. However, continued frequent consumption of dietary acids over time can lead to dental erosion [Bartlett, 2005]. In contrast, the damaging acids produced by bacteria locally on tooth surfaces associated with dental caries last in the oral environment for up to 60 min after exposure to fermentable carbohydrates.

The simplest way to protect the teeth from exogenic acid exposure is to refrain from excessive intake of potentially damaging acidic food and drinks. However, chang-

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C. Hjortsjö
Department of Prosthetic Dentistry and Oral Function
Institute of Clinical Dentistry, University of Oslo
PO Box 1109, Blindern, NO–0317 Oslo (Norway)
Tel. +47 2285 2368, Fax +47 2285 2344, E-Mail carl.hjortsjo@odont.uio.no

ing people's behavioural and dietary habits may be difficult and sometimes impossible. Therefore, an additional approach is required that provides preventive treatment that would help protect the teeth of people at risk of dental erosion and alleviate the symptoms associated with tooth tissue loss.

Using both in vitro and in situ studies, several authors have shown that acidic fluorides such as titanium tetrafluoride, stannous fluoride (SnF_2) and acidulated sodium fluoride are effective in reducing loss of enamel under acidic challenge [Büyükyilmaz et al., 1997; Hove et al., 2007, 2008; Schlueter et al., 2007, 2009]. Using an experimental in vivo model, toothpaste containing acidic SnF_2 has also been shown to reduce enamel dissolution greatly after 5 min compared with a neutral sodium fluoride toothpaste [Young et al., 2006]. Given that hydrofluoric acid (HF) is formed in fluoride solutions with low pH levels [Larsen and Jensen, 1986], it has been suggested that hydrogen and fluoride, as undissociated hydrogen fluoride, are important active components in these solutions [Brudevold et al., 1963]. In more recent experimental in vivo studies, SnF_2 and dilute HF solutions have shown promising results in reducing enamel dissolution following erosive challenge. Five minutes after a single 1-min treatment of the enamel, HF and SnF_2 solutions containing 0.03–0.1 mol/l fluoride successfully reduced enamel dissolution by 40–76% following a single citric acid challenge [Hjortsjö et al., 2009]. However, the duration of this erosion-preventive effect has not yet been tested. The aim of this study was therefore to evaluate the protective effect over time of a single treatment of enamel with aqueous solutions of SnF_2 and HF containing 0.1 mol/l fluoride, using the same experimental in vivo model as mentioned above. The hypothesis was that a single application of a SnF_2 or HF solution would not provide protection against enamel dissolution when re-challenged with citric acid after 1, 7, 14 or 28 days following the initial citric acid challenge.

Subjects and Methods

Subjects

The test subjects who took part in this study were either contacted by a recruiting firm (Norstat, Lillehammer, Norway) or by e-mail advertisement at the University of Oslo. The test subjects were recruited from the city of Oslo where the concentration of fluoride in the water is negligible and fluoride toothpaste is used by most persons. The participating subjects were in good general health, and relevant health questions were asked before starting the experiment. Prior to the study, a power analysis was performed in order to decide on the number of subjects (teeth) to be included in the study. With a desired power of 0.800, at the 5% level of significance, and an expected standard deviation of change of 0.300, this resulted in a sample size of 20 teeth. However, we expected a gradual loss of effect, and to meet this expectation we included more teeth than required. One hundred and four subjects, aged 18–57 years (mean age 26 years), were randomly assigned to eight groups according to test solution and evaluation time. Neither the test subjects nor the operator had any influence on the recruitment process. Written information about the study was given at the time of recruitment and again at the first meeting, and written informed consent was obtained prior to commencing the experiment. The protocol was approved by the National Committee for Research Ethics in Norway (REK-Sør: S-05223). Inclusion criteria were intact, non-filled healthy maxillary front teeth. Exclusion criteria were the presence of erosions, restorations, and any metal object on the front teeth (i.e., retainer or bonded jewellery, etc.). The subjects were not given any dietary or oral hygiene restrictions prior to or during the study.

Test Solutions, Teeth and Exposure Time

Two acidic fluoride solutions were tested in this study: 0.78% w/v SnF_2 (0.099 mol/l F) made from 99% tin II fluoride (Sigma-Aldrich Chemie, Steinheim, Germany) and 0.2% w/v HF solution (0.112 mol/l F) made from 40% hydrogen fluoride (Rectapur, Prolabo, Paris, France). The solutions were prepared fresh for each study day using deionized water to obtain the wanted concentration. One test solution was applied to each volunteer's teeth only once (one treatment/volunteer and tooth). Five teeth were excluded from the study prior to starting the experiment because it was not possible to isolate them according to the clinical protocol. The subject and tooth distribution is shown in table 1. The pH of the solutions measured at room temperature was 2.9 for SnF_2 (pH 90 WTW, Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) and 2.0 for HF (pH-meter Orion Star, Thermo Electron Corp., Beverly, Mass., USA).

Erosive Challenge

The teeth with intact pellicle were exposed to 5 ml 0.01 mol/l citric acid (0.18% w/v citric acid, pH 2.7, Sigma-Aldrich Chemie) applied using a peristaltic pump (Gilson model Minipuls 3, Villiers, France) at a flow rate of 6 ml/min and the acid was collected in a test tube before (etch I) and after (etch II) fluoride treatment. Etch II was carried out using the same procedure after 1 day, 7, 14 or 28 days.

Experimental Procedure

This study was a single-centre, randomized, single-blind study. The experimental in vivo procedure was the same as used in a recent study [Hjortsjö et al., 2009]. Prior to recruitment of test subjects, it was decided which test solutions were to be used on which test days. One operator (C.H.) with an assistant (G.J.) carried out all treatments on all test subjects who were given consecutive appointments following recruitment. Only one test solution was used throughout each day and in each person. The procedure was carried out in a dental chair at the Dental School Clinic. No attempt was made to polish the test teeth prior to the experiment, leaving them with a natural enamel pellicle and plaque present prior to testing. With the test subject lying down, the upper anterior part of the mouth was first isolated using two

Table 1. Calcium concentrations in the citric acid etch samples, the changes (ΔCa) and percent changes (%ΔCa) associated with the treatments

Test solution	Teeth/ subjects	Time days	Median etch I mg/l Ca	Median etch II mg/l Ca	Median ΔCa mg/l	Median %ΔCa	p value
HF	54/14	1	0.852 (0.707 to 1.27)	0.38 (0.285 to 0.590)	0.433 (0.223 to 0.802)[a]	54.1 (30.0 to 69.7)	<0.001
	53/14	7	1.08 (0.758 to 1.80)	0.739 (0.521 to 1.04)	0.291 (0.096 to 1.11)[a]	35.8 (13.5 to 55.3)	<0.001
	52/14	14	0.764 (0.440 to 1.30)	0.562 (0.377 to 1.09)	0.108 (–0.195 to 0.528)[b]	14.2 (–35.9 to 53.7)	0.116
	32/9	28	0.671 (0.475 to 0.933)	0.607 (0.414 to 1.00)	–0.036 (–0.239 to 0.285)[b]	–8.5 (–54.6 to 35.8)	0.837
SnF_2	56/14	1	0.508 (0.278 to 0.729)	0.686 (0.443 to 0.957)	–0.119 (–297 to 0.063)[a]	–24.2 (–95.9 to 8.4)	0.017
	52/13	7	0.513 (0.345 to 0.864)	0.672 (0.510 to 0.857)	–0.127 (–0.328 to 0.140)[a]	–26.02 (–70.7 to 23.6)	0.116
	52/13	14	0.489 (0.200 to 0.872)	0.771 (0.539 to 1.13)	–0.266 (–0.588 to 0.047)[a]	–62.28 (–168.8 to 13.0)	0.005
	48/12	28	0.604 (0.345 to 1.06)	0.735 (0.450 to 1.18)	–0.063 (–0.436 to 0.101)[a]	–10.2 (–89.4 to 17.9)	0.106

Medians with interquartile ranges in parentheses. For each type of fluoride, time periods sharing the same superscript letter are not significantly different from each other. Last column: significant differences between etch I and etch II.

cotton wool rolls placed in the maxillary sulcus and saliva suction in the lower jaw. Each tooth was then isolated using plastic strips (Odus Universal Strips, Odus Dental, Zurich, Switzerland) and light-bodied impression material (3M ESPE Permadyne Light Body, 3M ESPE, Seefeld, Germany) that was applied only to the palatal surfaces in order to prevent the test fluid from reaching that surface. The impression material was allowed to set (~6 min) before testing commenced. If possible, all four teeth (12, 11, 21, and 22) were isolated at the same time, and if not then pairwise (teeth 12, 21 and teeth 11, 22). The test subject was then raised to a sitting position and the erosive challenge was carried out by dripping the citric acid on each of the entire isolated labial tooth surfaces (etch I), one tooth at a time, using a peristaltic pump with a flow rate of 6 ml/min (Gilson model Minipuls 3, Villiers-le-Bel, France). The acid was collected directly into coded test tubes using a plastic funnel placed below the test teeth. The teeth were then rinsed gently with water for approximately 5 s using a triple syringe. The same test teeth and surfaces were then immediately exposed to either SnF_2 or HF solutions for 1 min using the same peristaltic pump (6 ml/min), and the test teeth were again gently rinsed for 5 s using a triple syringe. The subjects were then instructed not to eat or drink for 2 h. Both the rinsing water and the test solutions were collected and disposed of using a plastic cup in order to avoid any solutions being swallowed. The isolating plastic strips and impression material, cotton wool rolls and saliva suction were removed. The test subjects returned for the second acidic challenge (etch II) after either 1 day, 7, 14 or 28 days, as previously arranged.

The tooth isolation procedure was repeated prior to etch II. The same equipment and flow rate as used for etch I were then used for the second etch. The citric acid samples were again collected using a plastic funnel in coded test tubes and the teeth were rinsed for 5 s with water using a triple syringe. Isolation material, cotton wool rolls and saliva suction were again removed and the subject was asked to rinse the mouth thoroughly with water before rinsing with fluoride mouthwash (NaF 0.05%). The test subjects were also encouraged to rinse with a fluoride mouth wash for the following week in order to help remineralize any possible demineralized enamel. Acid etch samples were coded and stored at 4°C until analysis.

Calcium Analysis

Enamel dissolution of each tooth was examined by measurement of calcium concentration in the collected citric acid samples (etch I and etch II). An A Analyst 400 Atomic Absorption Spectrometer (Perkin Elmer Analytical Instrument, Norwalk, Conn., USA) was used for the analyses that were performed blind on the coded acid etch samples. Prior to analysis, lanthanum chloride (VWR International, Fontenay-sous-Bois, France) was added to the etch samples to a final concentration of 0.5% (1/10 lanthanum/sample) in order to counteract the negative effect of phosphorus on calcium sensitivity of the spectrometer.

Statistical Analysis

For each tooth the calcium concentration for etch I and etch II was determined and the change in calcium (ΔCa, mg/l) was calculated as etch I minus etch II. Median ΔCa (mg/l) was then determined for both test solutions. Percentage reduction in calcium loss (%ΔCa) was also determined for each tooth and median values were calculated for both test solutions. The Kolmogorov-Smirnov normality test failed and a non-parametric method was therefore chosen. For comparison of calcium loss before and after treatment for each test solution, a Wilcoxon signed-ranks test was performed. A Kruskal-Wallis one-way analysis of variance on ranks to determine difference between the groups and Dunn's multiple comparison between time periods for each fluoride type were used to compare their effect on calcium loss. The data were analysed using SigmaStat version 3.5 (Systat software, San José, Calif., USA). The differences were considered significant at the 0.05 level.

Results

The results are presented in table 1. A total of 411 healthy anterior teeth were tested in 104 subjects. The acid sample from one tooth was lost during storage (28-day HF group) and 11 samples were not included in the results after calcium measurements because no calcium was measured in either etch I or etch II (8 teeth in the 14-day HF group and 3 teeth in the 28-day HF group). A significant reduction in median calcium concentration for etch II was observed in three of the eight groups, all of which were HF groups. HF reduced enamel dissolution significantly after 1 day and 7 days ($p < 0.001$). After 14 and 28 days, the median effect was either not significant or below zero. Median %ΔCa for SnF_2 was below zero for all test periods.

Discussion

The hypothesis that a single application of SnF_2 or HF solution would not provide protection against enamel dissolution when re-challenged with citric acid after 1, 7, 14 or 28 days following the initial citric acid challenge was partly proven to be wrong. Under the conditions provided in this experimental in vivo study, a single application of the 0.2% HF solution was definitely shown to have a protective effect on enamel acid dissolution that lasted for at least 7 days. After 2 weeks, the protective effect could no longer be observed. Median percentage reductions in calcium dissolution indicated that some effect remained in the 14-day group but this was not statistically significant, as clearly shown in table 1. In a recent study testing acidic fluoride solutions using the same experimental model as in the present study, a mean reduction in calcium loss of 76% was recorded 5 min after a 1-min treatment with aqueous 0.2% HF [Hjortsjö et al., 2009]. This correlates well with the results in the present study, showing a reduction in effect during the first 24 h. In that same study, the SnF_2 solution (also containing 0.1 mol/l F) gave a mean reduction in calcium loss of 67% 5 min after a 1-min treatment. The present study indicates that the entire effect of the SnF_2 solution was lost during the first 24 h.

Compared with the effect of the dilute HF solution, the observed lack of effect of SnF_2 may be explained both by the low total fluoride concentration (0.1 mol/l F) and most likely also by the percentage of the fluoride present as hydrogen fluoride. In a previous study using the same experimental in vivo model, four consecutive 1-min treatments with commercially available SnF_2 toothpaste containing 0.10% F (0.055 mol/l F) resulted in only a 20% reduction of enamel dissolution 5 min after treatment [Young et al., 2006]. This was less than a third of the effect seen with a single application of aqueous SnF_2 solution, as mentioned above [Hjortsjö et al., 2009], and highlights the fact that the formulation of the fluoride plays an important role. In the present study, already after only 1 day, no protective effect was observed for SnF_2. The negative result after 1 day was significant and can only be interpreted as a lack of effect. This suggests that the results for SnF_2 for days 7, 14 and 28 are irrelevant. The fact that the lack of effect of SnF_2 was significant after 14 days is most likely a consequence of using statistics on 'negative data'. Given the nature of this experimental in vivo study, it is suggested that large individual physiological differences in the tooth enamel of the test subjects, and/or differences in the dietary and oral hygiene routines of these subjects will affect the results, but that this will also be the case in 'real life'.

Recent in vitro studies claim that tin is the ion that is mainly responsible for the reduction of enamel solubility in solutions containing tin and fluoride [Schlueter et al., 2009]. An experimental solution consisting of amine fluoride, sodium fluoride and a high concentration of tin chloride (2,800 mg/l Sn) reduced erosive tooth loss by 93% under severe erosive conditions compared to the positive control containing amine fluoride and 409 mg/l Sn. In the present study, however, one single application of the SnF_2 solution containing 5,909 mg/l Sn showed no effect after only 1 day.

In similar studies that have examined the effect of various preparations in reducing enamel dissolution in acids, the fluoride concentration and formulation vary considerably. In one in vitro study examining an SnF_2 solution with a fluoride concentration 5 times higher than what was used in the present study, a 91% reduction in enamel etching depth was observed [Hove et al., 2008]. Furthermore, the teeth in the present in vivo study were only exposed once to the fluoride treatment, and the test subjects carried on with their normal daily eating and oral hygiene routines until the second visit. Many recent in vitro and in situ studies involve cyclic de- and remineralization cycles in order to evaluate the erosion-inhibiting effects of acidic fluoride solutions. Using longitudinal radiography, a 10-day in vitro study comparing the effect of various fluoride solutions on enamel erosion caused by citric acid found that solutions containing SnF_2 were the most effective when applied 6 times daily after each etching [Ganss et al., 2008]. A 9-day in situ study involving twice-daily etching of enamel samples with HCl indicat-

ed that 3 applications of 0.5 mol/l fluoride in the form of either SnF_2 or TiF_4 solutions gave 91 and 100% protection, respectively, as measured by white light interferometry [Hove et al., 2008]. In an in vitro study testing increasingly severe erosive conditions for human enamel treated with a range of experimental solutions, those solutions containing high concentrations of tin gave the best effect under the most severe conditions [Schlueter et al., 2009]. In the present study, there was no control over how many acidic challenges the teeth were exposed to during the experimental period, but this can be expected to be comparable to the worst conditions outlined in the above study. The results of these studies therefore indicate that repeated treatments over a short period of time with HF or SnF_2 solutions, as tested in the present study, could be expected to provide an improved and possibly a longer-lasting protection of the enamel. Furthermore, an increase in the concentration of the SnF_2 solution is also likely to improve the effect. As there are many factors that play an important role in the oral cavity, this hypothesis needs to be tested in an experimental in vivo model.

In the present study, no negative control solution was included. A previous study showed that neutral sodium fluoride solution (0.1 mol/l F) did not prevent dissolution by citric acid 5 min after treatment [Hjortsjö et al., 2009]. It was therefore not considered ethical to expose further test teeth to such a control solution. The same holds true for the 0.31% TiF_4 solution (0.1 mol/l F) tested in that study, although one could expect that increasing the concentration of TiF_4 would improve the effect as seen in other studies [van Rijkom et al., 2003; Hove et al., 2008; Magalhães et al., 2008].

In a total of 11 test samples, no calcium was found in either etch I or etch II. These samples were excluded from the study since any change in calcium dissolution was impossible to calculate. Although the atom absorption spectrometer was calibrated before measurements, possible explanations for these results may be the physiological variations between test teeth, and the fact that errors may have been introduced when lanthanum was added to each sample before analysis. Ideally, triplicate calcium measurements for each tooth should have been performed. However, the small amount of citric acid etch used (5 ml) did not make this possible, and it was considered unethical to expose the test teeth to more citric acid than needed for one measurement only.

This previously used experimental in vivo model has several advantages over in vitro models. A natural pellicle is present prior to both the first and second etch, and it is possible to test the erosion-protective ability of different agents in an environment that goes a long way towards simulating the clinical situation. Furthermore, using each tooth as its own control helps to exclude large inter-individual test subject differences. However, the citric acid will almost certainly have a different effect on a pre-etched and fluoride-treated enamel surface (as is the case with etch II), compared to a natural, non-etched tooth surface as represented by etch I.

It can be concluded from this study that treatment of sound enamel with a 0.2% hydrogen fluoride solution had a protective effect against citric acid attack that lasted for at least 1 week. In contrast, the SnF_2 solution containing the same low fluoride concentration had no effect after only 1 day. Increasing the concentration of the SnF_2 solution may give improved and longer-lasting protection, and repeated treatments with these solutions may improve their effectiveness, but this remains to be tested in this in vivo experimental model.

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