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Abstract

OBJECTIVES: This study aimed to analyse the erosion-inhibiting potential of a single application of stannous chloride-containing fluoride solution on pellicle-covered enamel and dentine under constant acid flow conditions in vitro. DESIGN: Bovine enamel (n=60) and dentine (n=60) samples were exposed 1h to the oral cavity of 4 healthy volunteers to allow for in situ pellicle formation. Pellicle-covered samples were randomly assigned to three groups (each n=20 enamel and n=20 dentine samples; 5 enamel and 5 dentine samples/volunteer) and treated once with a SnCl2/AmF/NaF (800 ppm Sn(II), 500 ppm F, pH 4.5) or a NaF solution (500 ppm F, pH 4.5) for 2 min or remained untreated (controls). Samples were eroded with hydrochloric acid (pH 2.6) in a small erosion chamber at 60 microl/min for 25 min. Calcium release into the acid was monitored in consecutive 30s intervals for 5 min, then at 1 min intervals up to a total erosion time of 25 min using the Arsenazo III procedure. Data were statistically analysed by random-effects linear models (p<0.05). RESULTS: The stannous chloride-containing fluoride solution reduced calcium loss of enamel and dentine to up to 6 min and 3.5 min, respectively. Calcium loss (% of control) amounted from 24+/-7 (30s) up to 93+/-14 (6 min) in enamel and from 38+/-13 (30s) to 87+/-15 (3.5 min) in dentine. The sodium fluoride solution was unable to reduce enamel and dentine erosion at any time point. CONCLUSION: A single application of a stannous chloride-containing fluoride solution reduced enamel and dentine erosion up to 6 min and 3.5 min of constant acid flow, respectively.
Erosion-inhibiting potential of a stannous chloride-containing fluoride solution under acid flow conditions in vitro

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Protection of erosion by SnCl₂/AmF/NaF mouthrinse

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Results: The stannous chloride-containing fluoride solution reduced calcium loss of enamel and dentine to up to 6 min and 3.5 min, respectively. Calcium loss (% of control) amounted from 24±7 (30 s) up to 93±14 (6 min) in enamel and from 38±13 (30 s) to 87±15 (3.5 min) in dentine. The sodium fluoride solution was unable to reduce enamel and dentine erosion at any time point.

Conclusion: A single application of a stannous chloride-containing fluoride solution reduced enamel and dentine erosion up to 6 min and 3.5 min of constant acid flow, respectively.
Introduction

Given the fact that the occurrence of dental erosion is steadily increasing, the possible preventive measures for dental erosion have been widely investigated.\textsuperscript{1, 2} Although the efficacy of fluoride application in preventing dental erosion is still under debate, many studies have shown a protective effect of fluoride agents on erosion of enamel and dentine.\textsuperscript{3-9} Recent work demonstrated that fluoride preparations containing stannous ions showing promising protective effects against cyclic erosive attacks in enamel.\textsuperscript{3, 10} After the application of a tin-containing fluoride solution, a relatively dense tin-rich layer was present on enamel surface, thus reducing the erosive substance loss. Under cyclic erosive conditions, the tin-containing fluoride solution resulted in the incorporation of tin into enamel up to a depth ranged from 10 µm to 20 µm. However, no incorporation of tin into enamel was found in sound specimens.\textsuperscript{11}

Previous studies investigated the erosion-inhibiting potential of tin-containing fluoride solutions by cyclic de- and remineralization models over several days.\textsuperscript{16-12} In those studies, the solutions were applied frequently between multiple erosive attacks, probably leading to the formation of a relatively thick protective layer. However, so far, little is known about the duration of the protective effect of a single application of tin-containing fluoride solution against dental erosion. As the duration of the protective effect might give information about the frequency of application needed to ensure a constant protection, the preventive effect of a single application of a tin-containing fluoride solution on enamel and dentine erosion needs to be investigated. Further, to the authors’ knowledge, to date no study has investigated the effect of tin-containing fluoride on erosion progression in dentine.

Therefore, the aim of the current study was to investigate the erosion-inhibiting efficacy of a single application of a stannous chloride-containing fluoride solution on enamel and dentine erosion. The null hypothesis was that the erosive calcium loss of enamel and dentine, respectively, is not significantly different between fluoride-treated and control samples.
Materials and methods

Sample preparation and pellicle formation
Sixty enamel and 60 dentine specimens (3 mm diameter) were prepared from freshly extracted, undamaged bovine incisors and embedded in acrylic resin blocks 6 mm in diameter (Paladur, Heraeus Kulzer, Germany). The labial surfaces of the specimens were ground flat and polished with water-cooled carborundum discs (800-4000 grit, waterproof silicon carbide paper, Stuers, Erkrat, Germany). To prepare dentine cylinders, enamel was completely removed until dentine was just exposed. The samples were sterilized by γ-radiation (12 kGy, 4 h, Paul Scherrer Institute, Villigen, Switzerland). Before use, all specimens were kept in 100% humid atmosphere.

Fifteen enamel and 15 dentine samples were then randomly assigned to one of four healthy volunteers (1 female, 3 male, aged 27-32 y, without caries or periodontal diseases, physiological salivary flow rate, no medication taken during the study) for in situ pellicle formation. Sample size (five samples taken from each of 4 volunteers) was calculated accordingly to Brown and Prescott13 assuming that the standard deviation of the measurements is equal to 25%, α level equal to 5%, power equal to 80% and the correlation within each volunteer ρ is equal to 0.3.

Ethic approval for the study was granted by the local Ethics Committee (No 07/11). Participants received written instructions and gave their written consent. The participants presented physiological salivary flow rates (stimulated: >1 ml/min, unstimulated: >0.25 ml/min) and good oral health (no frank cavities or significant gingivitis/periodontitis). Custom-made acrylic palatal devices were provided with 8 buccal recesses in the areas of left and right maxillary second premolars and first molars for attachment of the samples. Each volunteer conducted four runs, wearing the maxillary appliance loaded with eight or seven randomly assigned samples. The position of each sample in the device was randomly determined for each volunteer. For standardization reasons, volunteers were instructed to use fluoridated toothpaste (1400 ppm fluoride, elmex CARIES PROTECTION, GABA,
Switzerland) for 7 days prior to and during the course of the experiment. Thereby, the volunteers were advised to brush their teeth twice daily in the morning and evening using the fluoridated toothpaste.

Fluoride solutions
Two different fluoride solutions were used: NaF (500 ppm F, pH 4.5, GABA, Therwil, Switzerland) and SnCl\textsubscript{2}/AmF/NaF (elmex Erosion Protection, 500 ppm F, 800 ppm Sn(II), pH 4.5, GABA, Therwil, Switzerland). The fluoride and tin content and pH of the solutions were checked prior to the experiment.

Experiment
Initially, all samples were stored in citric acid (1%, pH 2.3, 1 ml/ sample) for 60 s to induce a slight erosion as commonly found after consumption of an acidic drink. The samples were rinsed with tap water and inserted in the intraoral appliances. The appliances were worn for 1 h prior to the application of the test solutions to allow for the formation of a salivary pellicle, generally present on sample surfaces in vivo. The participants were instructed to refrain from consumption of any dietary products and oral hygiene treatment 1 h before insertion and while the appliances were in place.

Pellicle-covered enamel and dentine specimens (each n = 20 enamel and n = 20 dentine samples; 5 enamel and 5 dentine samples/ volunteer) were then extraorally once treated with one of the fluoride solutions for 2 min (1 ml/ sample, followed by 5 s rinsing with distilled water). The samples of the control group were treated with distilled water for 2 min.

Then the samples were subjected to erosion in a small erosion chamber, which was described previously by Wiegand et al.\textsuperscript{6} Briefly, each sample was fixed in a brass jig, which allowed exposure of the enamel surface to a small erosion chamber of 2 mm in diameter and 0.3 mm in height. Hydrochloric acid (2.5 mmol/L, pH 2.6) was pumped at 60 µl/min from a reservoir outside the chamber into the space erosion chamber. Eight chambers were each connected to a multichannel pump (Ismatec, Glattbrugg, Switzerland).
Samples were exposed to acid for a total of 25 min. For the first 5 min the acid was collected at consecutive 30 s intervals via an outlet pipe into a reservoir. The acid was then collected at consecutive 1 min intervals up to a total erosion time of 25 min.

Calcium analysis

The acid collected during the successive intervals was analysed for calcium using the Arsenazo III method (Fluitest, Ca-A-II, analyticon, Lichtenfels, Germany).\textsuperscript{14, 15} Arsenazo-III reacts with calcium in an acid solution to form a blue-purple complex. The intensity developed is proportional to the calcium concentration. Absorption was determined at $\lambda = 650$ nm.

Five microlitre of the acid solution were added to wells of microtitre plates containing 100 $\mu l$ of the reagent. Absorbance was read within 30 min after mixing of the solutions. Individual standard curves were obtained with standardized calcium solutions for determination of the calcium concentrations.

Statistical analysis

Calcium release into the acid was measured in each interval. Within each volunteer, calcium release of samples treated with SnCl$_2$/AmF/NaF solution or NaF solution, respectively, was calculated as percentage of the respective mean calcium loss of the control samples. Mean calcium loss (% of control) was computed for enamel and dentine groups.

To consider individual variation in pellicle formation or composition among the four volunteers, raw data were statistically analysed by random-effects linear models at each time point ($p < 0.05$, STATA, version 10.1, StataCorp, USA).

Results

Mean calcium loss (% of control) of enamel and dentine samples treated with SnCl$_2$/AmF/NaF or NaF is presented in figure 1.
SnCl$_2$/AmF/NaF decreased calcium loss of enamel significantly up to 6 min of erosion. Thereby, enamel erosion was reduced from $24 \pm 7\%$ after 30 s to $93 \pm 14\%$ after 6 min acid flow. In dentine samples, SnCl$_2$/AmF/NaF reduced erosion up to 3.5 min of acid flow. Calcium loss was reduced from $38 \pm 13\%$ after 30 s to $87 \pm 15\%$ after 3.5 min.

NaF was unable to reduce calcium loss in enamel and dentine samples significantly compared to control at any time point.

Discussion

The present experiment aimed to imitate a single application of stannous chloride-containing fluoride solution prior to an erosive attack like it might occur in a patient suffering from dental erosion. Initially, the samples were slightly eroded to imitate a tooth surface present after consumption of an acidic drink. Contrasting to previous studies using cyclic de- and remineralisation procedures with frequent applications of stannous-containing fluoride agents, the solution was applied only once in the present experiment to analyse the longevity of the erosion-protective effect of a single application. To simulate the clinical situation, the solutions were applied on specimens that were covered with an in situ formed pellicle. The pellicle was formed in situ as the composition of pellicle-like layers formed in vitro by immersion in artificial or human saliva might differ substantially from a native pellicle. The possible inter-individual variability of pellicle formation in the different volunteers was considered in the statistical analysis (random-effects linear models).

In the present study it was shown that the SnCl$_2$/AmF/NaF solution is able to reduce enamel and dentine erosion for several minutes of acid contact. The protective effect was most pronounced at the beginning and last for 3.5 min in dentine and 6 min in enamel. Previously it was shown that the erosion-protective potential of tin-containing fluoride solutions in enamel is related to the incorporation of tin in the outermost enamel layer. After application, a glaze layer possibly containing Sn$_2$OHPO$_4$, Sn$_3$F$_3$PO$_4$, Ca(SnF$_3$)$_2$ and CaF$_2$, is formed on the enamel surface. This layer is able to act as a protective barrier to the enamel capable of providing protection against erosive attack thus increasing the acid
resistance. In accordance with a previous study, the protective effect of SnCl₂/AmF/NaF solution was more durable in enamel than in dentine. It could be assumed that a similar mechanism like in enamel account for the effect of the tin-containing fluoride solution on dentine erosion showed that an extensive tin-rich deposit formed on dentine after application of stannous chloride, which occluded dentinal tubules. However, the different protective effect of tin-containing fluoride solution might be due to lower content of mineral in dentin, as the stannous ion is a potent reactant with hydroxyapatite. Moreover, it is not known so far whether the precipitates formed on dentin after application of tin-containing solutions are as acid-resistant as the precipitates formed on enamel.

Although the SnCl₂/AmF/NaF solution was most effective in the first minute of erosion and decreased thereafter, it must be taken into account that erosion was performed under rather severe conditions without counterbalancing of saliva. The constant acid flow prevented any saturation of the acid, which might take place under static conditions. Undoubtedly, constant exposure of acid flow will be highly unlikely to be reached during the normal consumption of acidic drinks. However, with this in vitro study it was important to simulate the worst case situation to obtain reliable data that would allow findings and recommendations applicable to a broad spectrum of patients. It is conceivable that the SnCl₂/AmF/NaF solution might be more effective in the clinical situation, where extrinsic or intrinsic acids are rapidly diluted and buffered by saliva, compared with this laboratory investigation.

In conclusion, the null hypothesis of this study was rejected as the calcium loss between test and control groups was significantly different. Under the limitations of the present study, it is assumed that that the SnCl₂/AmF/NaF solution provides a potential treatment option for patients suffering from enamel and dentine erosion. Further studies are needed to investigate whether the protective effect of the SnCl₂/AmF/NaF solution is stable against abrasion.
Acknowledgement

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Figure 1

Mean calcium loss (% of control) in enamel and dentin samples after treatment with NaF and SnCl₂/AmF/NaF
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