Efficacy of Fluoride Compounds and Stannous Chloride as Erosion Inhibitors in Dentine

C. Ganss\textsuperscript{a}  A. Lussi\textsuperscript{b}  N. Sommer\textsuperscript{a}  J. Klimek\textsuperscript{a}  N. Schlueter\textsuperscript{a}

\textsuperscript{a}Department of Conservative and Preventive Dentistry, Dental Clinic of the Justus Liebig University Giessen, Giessen, Germany; \textsuperscript{b}Department of Preventive, Restorative and Paediatric Dentistry, School of Dental Medicine, University of Bern, Bern, Switzerland

Key Words
Dentine \cdot Erosion \cdot Fluoride

Abstract
The aim of this study was to evaluate the anti-erosive effects of different fluoride compounds and one tin compound in the context of the complex pathohistology of dentine erosion, with particular emphasis on the role of the organic portion. Samples were subjected to two experiments including erosive acid attacks (0.05 molar citric acid, pH 2.3; 6 \times 2 \text{ min/day}) and applications (6 \times 2 \text{ min/day}) of the following test solutions: SnCl\textsubscript{2} (815 ppm Sn), NaF (250 ppm F), SnF\textsubscript{2} (250 ppm F, 809 ppm Sn), amine fluoride (AmF, 250 ppm F), AmF/NaF (250 ppm F), and AmF/SnF\textsubscript{2} (250 ppm F, 409 ppm Sn). The demineralised organic fraction was enzymatically removed either at the end of the experiment (experiment 1) or continuously throughout the experiment (experiment 2). Tissue loss was determined profilometrically after 10 experimental days. In experiment 1, the highest erosive tissue loss was found in the control group (erosion only); the AmF- and NaF-containing solutions reduced tissue loss by about 60%, reductions for SnCl\textsubscript{2}, AmF/SnF\textsubscript{2}, and SnF\textsubscript{2} were 52, 74 and 89%, respectively. In experiment 2, loss values generally were significantly higher, and the differences between the test solutions were much more distinct. Reduction of tissue loss was between 12 and 34% for the AmF- and NaF-containing preparations, and 11, 67 and 78% for SnCl\textsubscript{2}, AmF/SnF\textsubscript{2}, and SnF\textsubscript{2}, respectively. Stannous fluoride-containing solutions revealed promising anti-erosive effects in dentine. The strikingly different outcomes in the two experiments suggest reconsidering current methodologies for investigating anti-erosive strategies in dentine.

Recent research has drawn attention to the role of various fluoride compounds, particularly of polyvalent metal cations, in prevention and therapy of dental erosion. Compounds under study were titanium, hafnium, and zirconium fluoride, ferrous sulphate, stannous fluoride, and stannous chloride. Comparisons of these compounds, but also of sodium and amine fluoride, revealed significant differences with respect to their anti-erosive capacity in enamel [Ganss et al., 2008; Wiegand et al., 2008; Schlueter et al., 2009], but little is known about their role in dentine.

When dentine is exposed to erosive challenges under in vitro or in situ conditions, a histological feature appears which is characterised by a layer of demineralised organic material persisting on the surface [Ganss et al., 2009]. From clinical experience, however, it is unlikely...
that relevant amounts of organic material are retained on erosive dentine lesions in vivo. The difference in the histology of in vitro/in situ erosion and clinical erosion is of importance insofar as the general effect of fluoride probably is much less in the absence of this organic covering [Ganss et al., 2004]. The role of the organic matrix with respect to effects of various anti-erosive agents, however, has not been systematically investigated.

The aim of this study was to compare the anti-erosive effect of concentration-adjusted fluoride solutions, and one tin-containing solution, with particular emphasis on organic surface structures. The test solutions were stannous fluoride, sodium fluoride, amine fluoride, stannous chloride, and two commercially available mouth rinses containing amine fluoride/sodium fluoride and amine fluoride/stannous fluoride. The experiment was a cyclic model with and without continuous removal of organic components. The null hypothesis was that the fluoride compound has no impact on the anti-erosive effect.

Materials and Methods

Preparation of Dentine Specimens

Samples of coronal dentine were prepared from previously impacted human third molars (Exakt Abrasive Cutting System and Exakt Mikrogirnder, Exakt-Apparatebau, Norderstedt, Germany) and polished to P1200 (Leco, St. Joseph, Mich., USA). All grinding and polishing procedures were performed under copious water flow. Specimens were mounted on microscope slides (R. Langenbrinck, Teningen, Germany) with a light curing acrylic (Technovit 7230 VLC, Kulzer-Exakt, Wehrheim, Germany). One half of the experimental area was covered with the acrylic and served as a reference area for profilometry. The uncovered area was carefully checked for any remnants or contamination. Until use, specimens were stored in 100% humidity. After erosion, the acrylic was completely removed from the reference area with a scalpel, thoroughly checked again with a stereo microscope, and kept in 100% humidity at room temperature until further procedures were performed.

Solutions Used

Citric acid solution: 0.05 M citric acid monohydrate, pH 2.3. Remineralisation solution: 4.08 mM H3PO4, 11.90 mM NaHCO3, 20.10 mM KCl, and 1.98 mM CaCl2 [Gerrard and Winter, 1986], pH 6.7. Collagenase solution: 100 units collagenase/ml remineralisation solution overnight and between applications. In the control group, the samples were eroded 6 × 2 min per day and otherwise stored in the remineralisation solution. The samples were mounted on microscope slides placed in a rack that was transposed to the different solutions to ensure constant immersion times. The sample size was 20 per group.

Erosive demineralisation was performed by immersion in 250 ml citric acid for 6 × 2 min per day at room temperature under gentle agitation on a shaker (horizontal movements, frequency 35/min). Subsequently, the samples were rinsed with tap water for 1 min and afterwards immersed in the test solutions for 2 min. After immersion in test solutions, the samples were rinsed again with tap water for 1 min and stored in the remineralisation solution (experiment 1), or in the collagenase solution (experiment 2) to achieve a continuous enzymatic matrix digestion. The time between cycles was 1.5 h; samples were stored in the remineralisation solution overnight and between applications. In the control groups, the samples were eroded 6 × 2 min per day and otherwise stored in the remineralisation (experiment 1) or collagenase (experiment 2) solution. At the end of experiment 1, the organic material was removed by immersing the samples in the collagenase solution for 96 h at 37°C under constant agitation.

Tissue Loss Measurement

The profilometric procedure has previously been described [Ganss et al., 2007]. A Perthometer S8P (Perthen-Mahr, Göttingen, Germany) equipped with a contacting (FRW-750) pickup was used. For moisture control, the sample surface was covered with distilled water for 30 s prior to each tracing. The water was removed with absorbent tissue, without contact to the experimental area, immediately after the tracing was performed. The tracings were interpreted with Perthometer Concept 4.0 software (Perthen-Mahr). The vertical distance of the regression lines constructed on the reference and experimental areas was defined as tissue loss (in micrometres), and the loss was reported as the mean of three tracings per sample. Ten repeated analyses of a given sample yielded a standard deviation of 0.8 μm.
Fig. 1. Percent reduction of tissue loss compared to control after erosive demineralisation with citric acid (pH 2.3) and application of various fluoride and one stannous chloride solution. Light grey columns: demineralised organic tissue preserved during the experiment (experiment 1). Dark grey columns: demineralised organic material continuously removed during the experiment (experiment 2). Different letters indicate significant differences (upper-case letters, experiment 1; lower-case letters, experiment 2).

Discussion

The experiment was a basic research study using a relatively mild erosion model with frequent application of the test solutions. It must be emphasised that the study was not designed in view of clinical needs, but aimed to provide conditions balanced in a way to identify differences between the various compounds. Widely used fluorides (and combinations thereof) and one non-fluoride compound were included. The latter was SnCl$_2$ which, in enamel, has been shown to be as effective as sodium fluoride [Ganss et al., 2008].

For profilometry, contacting and non-contacting devices are available. The target criterion here was the loss of mineralised tissue, determined after removal of organic surface structures. To avoid measuring errors from some small organic remnants, a contacting device was used which pushes these soft structures aside thus resulting in a more valid value for mineral loss [Ganss et al., 2009].

In experiment 1, the demineralised surface zone was allowed to develop. When this structure increases in thickness, the pH at the demineralisation front would decrease to a lesser extent and dissolved mineral will be prevented from immediate removal by the surrounding liquid phase, thus increasing the state of saturation. These factors explain the finding that erosive mineral loss in dentine is non-linear [Hara et al., 2005; Ganss et al., 2009]. Finally, all solid mineral/liquid interactions will become diffusion-controlled. This would mean that, over time, (1) an active agent acts in a decreasingly acidic environment at the demineralisation front, and (2) it needs to diffuse through a collagen structure reaching considerable thickness.

To mimic the supposed character of in vivo lesions, the organic material was continuously removed in experiment 2. The consequence of this is that all processes stay surface-controlled, and the acid and test solutions interact directly with the mineralised tissue surface. From these considerations, it is not surprising that the tissue loss was much higher in all groups compared to experiment 1 (p ≤ 0.001 each). In the control group, a tissue loss of 117.4 ± 9.0 μm was found. Similar to experiment 1, all solutions reduced tissue loss significantly compared to the control (p ≤ 0.001 each). However, the effect of SnCl$_2$ (104.1 ± 8.4 μm), AmF (103.1 ± 8.9 μm), and AmF/NaF (97.6 ± 8.0 μm) was limited. NaF (77.8 ± 7.9 μm) reduced tissue loss by one third, whilst treatment with AmF/SnF$_2$ (39.3 ± 9.8 μm) or SnF$_2$ (26.2 ± 3.6 μm) was most effective (fig. 1).

Results

Experiment 1

The largest tissue loss was found in the control group (52.3 ± 9.1 μm). Compared to the control, all solutions reduced tissue loss significantly (p ≤ 0.001 each). Loss values halved after application of SnCl$_2$ (25.0 ± 5.8 μm), NaF (21.4 ± 3.0 μm), AmF (20.1 ± 4.1 μm), and AmF/NaF (19.9 ± 1.7) treatments gave similar results. Treatment with AmF/SnF$_2$ resulted in a tissue loss of 13.6 ± 2.4 μm. The most effective solution was SnF$_2$ (5.6 ± 4.1 μm), reducing the tissue loss almost completely (fig. 1).

Experiment 2

After continuous matrix digestion, tissue loss was much higher in all groups compared to experiment 1 (p ≤ 0.001 each). In the control group, a tissue loss of 248–252

Ganss/Lussi/Sommer/Klimek/Schlueter
loss in experiment 2 was generally much higher than in experiment 1.

However, the demineralised matrix must exhibit also specific effects. In the presence of the collagen structures, the fluoride compound appeared to play only a minor role, as far as NaF and AmF are concerned; the three respective test solutions all reduced tissue loss by about 60%. This is in contrast to findings in enamel, where AmF, AmF/NaF, and NaF solutions reduced tissue loss on the range of 15, 43, and 73%, respectively. It was supposed that in contrast to the cation in NaF, the amine part must have had some adverse effects [Ganss et al., 2008], which obviously had become less relevant in the presence of the organic matrix. When the organic dentine portion was continuously digested, similar effects as in the enamel could be assumed. Indeed, in the second experiment, AmF, AmF/NaF, and NaF solutions reduced tissue loss in the range of 12, 17, and 34%, respectively. These effects were less distinct compared to enamel, but the differences between the fluoride compounds were in a similar proportion.

Besides the fluoride ion, the tin ion is an anti-erosive agent. Several studies have shown promising effects of stannous fluoride [Willumsen et al., 2004; Hove et al., 2006, 2008; Ganss et al., 2008] or tin- and fluoride-containing solutions [Schlueter et al., 2009] in enamel. This has now also been confirmed for dentine.

However, more distinct effects from these solutions could have been expected in experiment 1. Even if the main histological structure of the demineralised tissue is preserved, alterations at a molecular level can occur also under short-term applications of relatively mild acids [Eliades et al., 1997]. Further, the storage in the remineralisation solution might have had effects on non-collagenous proteins [Lussi and Linde, 1993; Clarkson et al., 1998], which play an important role with respect to interactions of inorganic ions with the organic matrix [Linde, 1989]. The effects of acid exposure and storage in the remineralisation solution might have induced alterations that result in remnants of phosphophoryn [Lussi et al., 1988; Linde and Lussi, 1989] probably responsible for modifying the diffusion of ions selectively and filtering the positively charged tin ions to a greater extent than the negatively charged fluoride ions.

In experiment 2, distinct differences between the SnF₂-containing solutions and the AmF and NaF preparations, as well as the SnCl₂ solution were found. The relative effects observed were comparable to the findings in enamel. To date, little is known about the mode of action of Sn and F in dentine. SEM studies on pre-etched dentine have shown that treatment with a 2% aqueous SnF₂ solution results in the deposition of Sn and F in the form of a continuous layer of globules [Ellingsen and Rølla, 1987]. Similar to enamel, these structures must be assumed to be relatively acid-resistant. As the tin ion alone had only limited effects, it must be the combined action of tin and fluoride that causes the beneficial results in dentine.

In conclusion, the null hypothesis is rejected as in both experiments significant effects were found. In the presence of the organic matrix, all test solutions showed considerable anti-erosive effects. Even if significant, however, the differences between the various compounds were not very distinct. When the organic matrix was continuously removed, the tissue loss generally increased, and the various compounds revealed markedly different anti-erosive properties. Considering these experimental outcomes and the fact that organic material probably will not persist on the surface of erosive lesions in relevant amounts in vivo, it appears necessary to reconsider current in vitro and in situ methodologies.

Acknowledgement

We thank GABA International, Therwil, Switzerland for providing the AmF solution, Meridol mouth rinse, and Elmex anticaries dental rinse.

References


Fluoride Compounds and Dentine Erosion

Caries Res 2010;44:248–252

251


Willumsen T, Øgaard B, Hansen BF, Rolla G: Effects from pretreatment of stannous fluoride versus sodium fluoride on enamel exposed to 0.1 M or 0.01 M hydrochloric acid. Acta Odontol Scand 2004;62:278–281.