Acta Odontologica Scandinavica, 2010; 68: 180–184

informa
healthcare

ORIGINAL ARTICLE

Tin and fluoride as anti-erosive agents in enamel and dentine in vitro

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Abstract

Objective. To investigate the efficacy of an experimental tin- and fluoride-containing mouth rinse on progression of erosion in enamel and dentine in vitro. ***Material and methods.*** Human enamel and dentine specimens were subjected to a cyclic demineralization and remineralization procedure for 10 days, with six 5-min demineralization periods per day. Erosive demineralization was performed with 0.05 M citric acid (pH 2.3). Except in the negative control group, the specimens were treated for 2 min with mouth rinses after the first and sixth demineralizations. An experimental tin-containing fluoride mouth rinse [125 mg/kg F^- (amine fluoride), 375 mg/kg F^- (NaF), 800 mg/kg Sn^{2+} ($SnCl_2$)] and an experimental sodium fluoride mouth rinse (500 mg/kg F^-) were used (both pH 4.5). A commercially available, tin-containing mouth rinse served as a positive control (pH 4.2, 409 mg/kg Sn^{2+}, 250 mg/kg F^-). Tissue loss was determined profilometrically. ***Results.*** The highest tissue loss was found in the negative control group, in both enamel and dentine. In enamel, the NaF solution showed almost no effect. Both tin-containing solutions significantly reduced tissue loss (positive control: 65%; 800 mg/kg Sn^{2+}: 78%; both $p \leq 0.001$ compared to negative control). In dentine all mouth rinses significantly reduced tissue loss (positive control: 43%; 800 mg/kg Sn^{2+}: 53%; NaF: 40%; all $p \leq 0.001$ compared to negative control). ***Conclusions.*** In enamel, the efficacy of mouth rinses depended on the compound used; tin-containing preparations were notably effective. In dentine, however, reduction of substance loss was nearly the same in all treatment groups.

Key Words: *Dentine, enamel, erosion, fluoride, tin*

Introduction

Restorative therapy for dental erosion is often difficult, expensive, and time-consuming. Hence, restorative measures are the second choice for therapy and can be avoided by using suitable non-invasive therapeutic methods. Generally, erosion stops if the cause is eliminated; therefore, the therapy is predominantly causally oriented. However, in cases with persisting acid challenges, a supporting symptomatic therapy is necessary. Sometimes, the frequent use of fluoride preparations, like mouth rinses, is recommended [1], even though there is no consensus regarding their efficacy. It is thought that such measures should modify the tooth surface, resulting in a reduced solubility of the dental hard tissue in acids and a reduced loss of surface hardness. Recent studies have shown that tin-containing preparations are efficient as anti-erosive agents in enamel both in vitro [2–6] and in situ [4,7]. The majority of studies used preparations with relatively high concentrations of tin, which are more suitable for professional application. Since patients with erosions are often exposed to chronic and frequent acid challenges, a measure for daily home application would appear meaningful. In this context, studies revealed that tin-containing preparations with a concentration in the upper range (1000 mg/kg F^-; $\approx$ 2000 mg/kg Sn^{2+}), which are suitable for over-the-counter products, were promising in terms of their anti-erosive potential [5,7]. In situ application, however, has shown that side-effects such as an astringent feeling on the mucosa and a dull feeling on the teeth may occur [7]. Therefore, reduction of the concentration of the active agents to a level at which fewer side-effects could be expected is recommended.

Regarding dentine erosion, there is limited knowledge about the efficacy of fluoride measures. In cases with severe erosion, however, it is characteristic that

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(Received 29 October 2009; accepted 14 December 2009)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016350903555395

dentine is exposed and, therefore, patients with distinct erosive defects would benefit from a therapeutic approach which is effective in enamel as well as in dentine. Furthermore, studies reveal that tin-containing fluoride solutions, potentially applicable as a mouth rinse, are most effective in enamel, although this has not yet been proven for dentine. The histology of dentine erosion is distinctly different from that of enamel erosion. Enamel is mainly composed of minerals and the impact of acids leads to bulk substance loss, followed by a small band of partially demineralized enamel [8]. In enamel, erosion is predominantly a surface-controlled process. Dentine, however, also contains a great amount of protein, mainly collagen. This organic fraction is exposed after an erosive demineralization, can reach considerable thickness [9] and remains on the surface [10], whilst the demineralization continues below the surface. Thus, in dentine, erosion progression is predominantly a diffusion- and not a surface-controlled phenomenon. Consequently, the results for enamel cannot simply be translated to dentine.

The aim of this study was to compare the efficacy of an experimental tin-containing fluoride mouth rinse [amine fluoride (AmF)/NaF/$SnCl_2$] and an experimental sodium fluoride mouth rinse with clinically meaningful concentrations of active agents in both enamel and dentine. Both solutions were adjusted with respect to fluoride concentration (500 mg/kg F^-) and pH (4.5). A commercially available tin- and fluoride-containing mouth rinse (AmF/SnF_2) was used as a positive control. The null hypothesis was that there was no difference between the stated solutions.

Material and methods

Specimen preparation

From 30 freshly extracted, previously completely impacted human third molars without cracks, 80 longitudinal enamel and 80 longitudinal dentine specimens were prepared. The natural surfaces of enamel specimens and the surfaces of dentine specimens were ground flat and polished under sufficient water flow (50 ml/min; Exakt Abrasive Cutting System and Exakt Mikrogrinder; Exakt-Apparatebau, Norderstedt, Germany; P800 and P1200 silicon carbide abrasive paper; Leco, St. Joseph, MI, USA), resulting in an experimental area of at least 3 mm × 3 mm. Dentine and enamel specimens were randomly divided into four groups ($n = 20$ each) and 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 of each specimen was covered with the acrylic and served as a reference area for profilometric measurement. Prior to treatment, specimens were inspected under a microscope (magnification 10×; SMZ-1, Zoom Stereomicroscope; Nikon GmbH, Düsseldorf, Germany) to ensure that there was no contamination in the experimental area. All specimens were stored under conditions of 100% humidity until use.

Treatment

All specimens were subjected to a cyclic demineralization and remineralization procedure with six 5-min demineralization periods per day. For erosive demineralization, a 0.05 M citric acid solution (pH 2.3, citric acid monohydrate; Merck, Darmstadt, Germany) was used. The demineralization and remineralization cycles were performed over a total of 10 days. In all groups, except the negative control groups, specimens were treated with the (experimental) mouth rinses for 2 min each after the first and last demineralization periods. The composition of the solutions is shown in Table I. The NaF and the 800 mg/kg Sn mouth rinse were adjusted to pH 4.5; the positive control mouth rinse had a pH of 4.2.

Prior to transfer to the next solution, the specimens were rinsed for 1 min with tap water. Inbetween fluoride treatments and demineralization in the negative control group, as well as overnight, specimens were stored in a remineralization solution [11] consisting of 4.08 mmol/l H_3PO_4, 20.10 mmol/l KCl, 11.90 mmol/l Na_2CO_3, and 1.98 mmol/l $CaCl_2$ with a pH of 6.7 (all chemicals from Merck). All solutions were renewed at the beginning of each experimental day; the pH of all solutions was controlled at the beginning and end of each experimental day. Dentine and enamel specimens were treated in the same containers filled with 250 ml of the respective solution; all groups were treated simultaneously. All procedures were performed under gentle agitation at room temperature.

Table I. Definition of groups and compositions of the mouth rinses.

Group	Composition of the solution
Negative control	No solution used
Positive control	125 mg/kg F^- as AmF[a] (0.16% w/w)
	125 mg/kg F^- as SnF_2 (0.05% w/w)
	409 mg/kg Sn^{2+} as SnF_2
800 mg/kg Sn	125 mg/kg F^- as AmF[a] (0.16% w/w)
	375 mg/kg F^- as NaF (0.08% w/w NaF)
	800 mg/kg Sn^{2+} as $SnCl_2$ (0.15% w/w $SnCl_2$)
NaF	500 mg/kg F^- as NaF (0.11% w/w NaF)

[a]Olaflur, GABA International AG, Therwil, Switzerland.

Tissue loss measurement

Tissue loss was measured profilometrically after the last experimental day. The measure of interest was the step height between the surface of the reference area and the demineralization front on the experimental area. In dentine, the exposed organic fraction was therefore removed after the last experimental day prior to measurement [12,13] with collagenase solution [96 h at 37°C; 100 U/ml remineralization solution; collagenase from *Clostridium histolyticum* type VII (Sigma Aldrich, St Louis, MO, USA), with a collagen digestion activity of 1680 U/μg of solid at 25°C and pH 7.5 in the presence of calcium ions]. The acrylic cover was carefully removed, and the surfaces were checked for acrylic remnants or damage. In dentine, all procedures were performed with thorough moisture control. In both substrates, measurements were performed with a Perthometer S8P (Mahr, Göttingen, Germany). Three traces were made on each specimen at intervals of 0.25 mm, each 1.75 mm in length, which were interpreted with special software (Perthometer Concept 4.0; Perthen Mahr, Göttingen, Germany). The vertical distance between the midpoints of the regression lines on the reference and the experimental area on each trace was defined as tissue loss and was calculated by the software. Repeated analysis, when removing and repositioning the sample in the system (10 repeated analyses of one sample), resulted in a standard deviation (SD) ± 0.8 μm in cases of distinct substance loss and ± 0.9 μm in cases of slight substance loss. The repeated analysis of one trace showed a SD of ± 0.1 μm. The variance of profiles of untreated specimens was 0.3 ± 0.5 μm.

Statistics

The statistical analysis of data was performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). The Kolmogorov–Smirnov test was used for checking the normal distribution of the data. A significant deviation from homogeneity of variances was found (Levene's test); therefore, the comparison of groups was performed with an ANOVA and Tamhane's post-hoc test. The level of significance was set at 0.05.

Results

Quantitative results [mean (SD); microns] and reduction relative to control group (%) are given in Table II.

In enamel, tissue loss was highest in the negative control group. In contrast to NaF, both tin-containing solutions reduced tissue loss significantly. The experimental 800 mg/kg Sn mouth rinse reduced tissue loss to a significantly greater extent than the positive control mouth rinse with the lower concentration.

In dentine, however, all fluoride solutions significantly reduced tissue loss. The best reduction was obtained with the application of the experimental 800 mg/kg Sn mouth rinse. No difference was found between the positive control and the NaF mouth rinse.

Discussion

This in vitro experiment should mimic the acid challenges which can occur in patients at high risk for dental erosion, e.g. vegetarians or people who frequently consume acidic soft drinks [14,15]. The 800 mg/kg Sn solution was chosen because it showed the best results for enamel in a previous study [16] and thus was also tested in dentine. At pH 4.5, tin-containing solutions are not stable. Therefore, AmF was added to the experimental solution, as it has stabilizing properties [17]. In a previous in vitro study [2], it has been shown that NaF is more effective than AmF as an anti-erosive agent; therefore NaF was used as an additional source of fluoride ions. Independent sources of tin and fluoride were used to provide the most effective ratio between fluoride and tin, independent of the other components. An experimental NaF mouth rinse with the same pH and fluoride concentration was tested as this fluoride compound is mostly contained in commercially available mouth rinses, is often used in the literature as a reference and is free of polyvalent metal cations. Thus, the impact of the stannous ion on the erosion-inhibiting efficacy of mouth rinses could be investigated, which was of particular interest for dentine. A commercially available tin-containing mouth rinse, which contained half

Table II. Tissue loss [mean (SD)] and reduction of tissue loss relative to the control group in enamel and dentine after 10 days of cyclic de- and remineralization. Groups without statistically significantly different results are indicated by the same superscripted letters.

	Enamel		Dentine	
Group	Tissue loss (μm)	Reduction relative to negative control (%)	Tissue loss (μm)	Reduction relative to negative control (%)
Negative control	74.1 (12.1)[a]		92.6 (8.8)[a]	
Positive control	26.3 (7.2)[b]	65	52.7 (6.1)[b]	43
800 mg/kg Sn	16.6 (5.9)[c]	78	43.6 (4.6)[c]	53
NaF	69.5 (13.7)[a]	6	55.9 (3.6)[b]	40

the concentrations of the active agents tin and fluoride, was used as a positive control.

Regarding the results in enamel, the highest tissue loss values were obtained, as expected, in the negative control group. The NaF mouth rinse showed a reduction in tissue loss of only 6% and was not effective, whereas a reduction in tissue loss of 65%–78% was achieved by the application of the tin-containing solutions. Comparable results were achieved by Hove and co-workers [3,18], Hooper et al. [19], and Wiegand et al. [20], who compared the efficacy of tin-containing fluoride preparations and NaF preparations adjusted to the same fluoride level.

The differences in efficacy of the NaF and tin-containing mouth rinses are probably due to differences in the reaction modes of the various compounds. NaF forms CaF_2-like precipitates on the tooth surfaces, as shown by various microscopic and analytical techniques [21]. These deposits are readily dissolvable at acidic pH and show, particularly in vitro, only low resistance to acid impacts. Therefore, the effect of NaF as an anti-erosive agent on enamel in vitro is limited. In contrast, stannous and fluoride ions in combination can react in many ways with the tooth surface. It is well known that the application of tin-containing fluoride solutions leads to deposits on the tooth surface [22,23], and there are indications that these deposits are relatively resistant to acids [2,3]. It is also known that the stannous ion reacts with pure hydroxyapatite [24,25] and with the surface of the dental hard tissue [22,26], resulting in reduced hydroxyapatite or enamel solubility [2,17,27]. A recent study [28] has shown that tin is not only retained on the tooth surface but can also be incorporated into eroded enamel, forming structurally modified and less acid-soluble surface zones. That study has also shown that the quantity of incorporation depends on the concentration of tin in the solution. A higher tin concentration leads to a higher uptake of tin and to better efficacy [28]. As in that study, the experimental mouth rinse with the higher tin concentration used in the present study led to a better reduction in tissue loss than the positive control solution containing half the tin concentration.

Regarding the results of the present study in dentine, the differences between the different mouth rinses were generally small and, except for the NaF mouth rinse, the efficacy of the tin-containing formulations was considerably less than in enamel. The effect of the fluoride-containing solutions was virtually independent of the type of fluoride compound. Similar results have been found in studies that have compared the erosion-inhibiting effect of TiF_4 solutions with NaF solutions in dentine [29,30]. The lack of differences might be a result of the histological differences between enamel and dentine, at least in vitro. An acid impact leads to exposure of the organic fraction in dentine. This matrix plays a decisive role in the development of dentine erosion in vitro and can act as a diffusion barrier [31,32], slowing down the progression of erosive mineral loss. This slowing down is probably a result of an increase in pH at the demineralization front. This can also have an impact on the efficacy of fluoride compounds. Under very mild conditions, NaF is able to distinctly reduce erosive tissue loss in enamel [2], but not under severe, more acidic, conditions [3]. Perhaps as a result of an increase in pH at the demineralization front, NaF showed a more protective effect in dentine than in enamel. However, it could also be that not only did the pH of the acid increase during diffusion through the organic matrix but also the pH of the mouth rinses. It is known that fluoride solutions show less efficacy with increasing pH [20,30,33]. This could be one reason for the lower efficacy of the tin-containing mouth rinses in dentine compared to enamel.

Of course, the matrix acts as a diffusion barrier not only for acids but also for active ingredients, which could be another reason for the leveling out of the efficacy of the different compounds. The matrix contains several proteins and proteoglycans, which are predominantly negatively charged [34]. The negative charge could affect the positively charged cations (e.g. stannous and sodium ions). Therefore, only a fraction of these ions would reach the demineralization front. In the case of active cations, like the stannous ion, notable differences in efficacy between enamel and dentine would therefore occur, and the efficacy of tin with fluoride and NaF in dentine would more or less be the same. However, it is still not clear whether an organic matrix with considerable thickness develops in vivo and whether these mechanisms can play a role under clinical conditions. Further studies investigating in vivo dentine erosion could give more information.

One can conclude from the results of this study that the efficacy of an anti-erosive mouth rinse in enamel depends on the cation of the fluoride compound used. Both tin-containing mouth rinses were effective, with their efficacy depending on the concentration of the active agents. In dentine, however, the erosion-inhibiting efficacy was less dependent on the compound, at least under the in vitro conditions used and in the presence of a thick organic matrix.

Acknowledgement

The study was supported by GABA International AG, Therwil, Switzerland.

Declartion of interest: The authors state that no conflict of interest exists.

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