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In vitro efficacy of experimental tin- and fluoride-containing mouth rinses as anti-erosive agents in enamel

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ABSTRACT

Objectives: The aim of this in vitro study was to investigate the efficacy of various experimental tin- and fluoride-containing mouth rinses with stepwise reduced concentrations of the active agents on erosion progression in enamel.

Methods: Human enamel specimens were subjected to a cyclic demineralisation and remineralisation procedure for 10 days with 6 demineralisation periods per day, 5 min each. Erosive demineralisation was performed with 0.05 M citric acid (pH 2.3). Except in the control groups, the specimens were treated for 2 min with experimental mouth rinses after the first and sixth demineralisations. The tin concentrations ranged between 800 and 2800 ppm, and fluoride concentrations of 500 and 250 ppm were used. All preparations were adjusted to pH 4.5. As positive control, a commercially available, tin-containing mouth rinse was used (pH 4.2, 409 ppm Sn^{2+}, 250 ppm F^-). Tissue loss was determined profilometrically.

Results and conclusion: As expected, the highest tissue loss was found in the negative control group. All experimental mouth rinses were able to reduce tissue loss significantly ($p \leq 0.001$). The best reduction was achieved by the 2800 ppm Sn^{2+}, 500 ppm F^- solution (80%). The lowest reduction was achieved by the 800 ppm Sn^{2+}, 250 ppm F^- solution (54%). Amongst the 500 ppm F^- solutions, in the Sn^{2+} concentration range of 2800–800 ppm, only small differences in efficacy were observed, meaning that the tin concentration can probably be reduced without losing efficacy. This factor is particularly important if one regards the possible clinical applicability of such mouth rinses.

1. Introduction

Enhancing the resistance of tooth surfaces against acids is the central point of a symptomatic therapy for dental erosions. Several therapeutic approaches have been discussed. One possibility is the coating of the dental surfaces with dentine adhesive, but with the shortcomings that the patient is reliant on his dentist for this measure and that such coatings show very limited long-term success.[1] Alternatively, the regular and frequent application of fluoride preparations is recommended,[2] albeit no consensus exists on their effectiveness. Results for the protective effects of the topical fluoride application are variable and range from no or limited effects[3,4] up to a nearly complete inhibition of erosive mineral loss.[5,6] Usually, common fluoride preparations, such as sodium or amine fluoride, are used for therapy. The effect of these fluorides is attributed to the formation of CaF_2-like layers on the tooth surface, which should act as a shield against acids. Since the thickness of the CaF_2-like layers increases due to prolonged application durations and preparations with high

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fluoride concentration, one has assumed that the efficacy of fluoride measures predominantly depends on the intensity of the fluoridation, which was seemingly confirmed by several studies.[7,8] One major problem with such intensive fluoridations is that the procedure is very time consuming and expensive, thus, it can only be limitedly recommended as a long-term therapy. Therefore, recent studies investigated fluoride compounds other than sodium or amine fluoride that contained polyvalent metal cations[9–14] to find other, possibly more effective, agents against erosive substance loss. In this context, the stannous ion has shown very good anti-erosive properties.[5,6,10,15–17] Interestingly, most of the cited studies investigated the efficacy of tin also with the assumption that high doses of fluoride preparations are more effective, so very concentrated solutions were used (up to 9500 ppm F^- and up to 30,000 ppm Sn^{2+}).

In a recent in situ study, using a relatively high-dose F-/Sn-solution (AmF/NaF/$SnCl_2$: 1000 ppm F^-, 1900 ppm Sn^{2+}), we have shown that this solution was notably effective with a 73% reduction in tissue loss compared to an NaF solution (28% reduction) even under severe erosive conditions. However, most participants reported an astringent feeling on the mucosa and an unpleasant, dull feeling on the tooth surfaces after the use of the solution. These sensations were, amongst others, ascribed to the high content of tin, since polyvalent metal salts like aluminium sulphate, zinc chloride and stannous chloride can lead to an astringent feeling.[18,19] Therefore, a reduction of the active components whilst maintaining a good level of efficacy would be worthwhile.

The aim of this in vitro study was, therefore, to compare the efficacy of various pH-adjusted AmF-/NaF-/$SnCl_2$-containing experimental mouth rinses with stepwise reductions in the Sn^{2+} and F^- concentrations to reduce the erosive tissue loss in cyclic demineralised and remineralised enamel. The null hypothesis tested was that there is no difference between the various mouth rinses.

2. Materials and methods

2.1. Specimen preparation

One hundred and thirty-two longitudinal enamel specimens were prepared from 45 freshly extracted, completely impacted human third molars without cracks. All donors lived in an area with ≤0.03 ppm fluoride in the drinking water. The natural surfaces of specimens were ground flat and polished under sufficient water flow (Exakt Abrasive Cutting System and Exakt Mikrogrinder, Exakt-Apparatebau, Norderstedt, Germany; P800 and P1200 silicon carbide abrasive paper, Leco, St. Joseph, USA). The preparation resulted in an experimental area of at least 3 mm × 3 mm. The specimens were randomly divided into 11 groups ($n = 12$ each) and mounted on microscope slides (R. Langenbrinck, Teningen Germany) with a light-curing acrylic (Technovit 7230 VLC, Kulzer-Exakt, Wehrheim, Germany), with six specimens per slide. One half of the experimental area of each specimen was covered with acrylic. This area served as a reference area for profilometric measurement. After covering, the specimens were scrutinised under a microscope (magnification 10×, SMZ-1, Zoom Stereomicroscope, Nikon GmbH, Düsseldorf, Germany) to ensure that there were no acrylic contaminations in the experimental area. The specimens were stored in 100% humidity until use.

2.2. Treatment

The study was divided in two experiments with the same procedures. In experiment 1, experimental mouth rinses with high tin concentrations were used; in experiment 2, the tin concentrations were reduced. All specimens were subjected to a cyclic demineralisation and remineralisation procedure with six demineralisation periods per day for 5 min each. For erosive demineralisation, a 0.05 M citric acid solution (pH 2.3, anhydrous citric acid, Merck, Darmstadt, Germany) was used. The demineralisation and remineralisation cycles were performed over a total of 10 days. In all groups, except in the negative control groups, specimens were treated with the (experimental) mouth rinses after the first and last demineralisation periods, for 2 min each. In both experiments, seven different experimental fluoride- and tin-containing mouth rinses and one commercially available mouth rinse as a positive control were tested (for details see Table 1). All experimental mouth rinses were adjusted to pH 4.5 and contained stabilisers and other additives.[a] Slides with specimens were put into special racks that were then transferred to a container filled with 250 mL of the respective solution to achieve a constant immersion time. Prior to the transfer to the next solution, the specimens were rinsed for 1 min with tap water. During the remaining time, the specimens were stored in a remineralisation solution[20] (4.08 mM H_3PO_4, 20.10 mM KCl, 11.90 mM Na_2CO_3, and 1.98 mM $CaCl_2$ (all chemicals from Merck), with a pH of 6.7). The solutions were renewed at the beginning of each experimental day, and the pH of all solutions was controlled at the beginning and at the end of each experimental day. All groups of one experiment were treated simultaneously. All procedures were performed under gentle agitation at room temperature.

2.3. Tissue loss measurement

Tissue loss was measured profilometrically after the last experimental day of each experiment. The acrylic cover was carefully removed, and the surfaces were checked for acrylic remnants or damage. The measurements were performed with a Perthometer S8P (Mahr, Göttingen, Germany) equipped with an optical stylus (Focodyn, Rodenstock, Munich, Germany). On each sample, three traces, each of 1.75 mm in length, were made at intervals of 0.25 mm. The traces were interpreted with special software (Perthometer Concept 4.0, Perthen Mahr, Göttingen, Germany). Two regression lines were constructed on each trace. The first one on the reference area was 0.4 mm in length, and the second one on the experimental area was 0.6 mm in length; both lines were at a distance of 0.3 mm from the edge between the reference and experimental areas. The mid-points of both regression lines were calculated by software. The vertical distance between the mid-points was defined as tissue loss.

[a] Formulated by GABA International AG, Therwil, Switzerland.

Table 1 – Compositions of all mouth rinses; except positive control all experimental mouth rinses were adjusted to pH 4.5.

Group	Total fluoride content (ppm)	Compound	Fluoride concentration (ppm)	Tin concentration (ppm)
Experiment 1				
Positive control (pH 4.2)[a]	250	0.16% (w/w) amine fluoride[b] 0.05% w/w SnF_2	125 125	409
Group 1.1	500	0.19% (w/w) amine fluoride[b] 0.08% (w/w) NaF 0.53% (w/w) $SnCl_2$	150 350	2800
Group 1.2	500	0.19% (w/w) amine fluoride[b] 0.08% (w/w) NaF 0.4% (w/w) $SnCl_2$	150 350	2100
Group 1.3	500	0.16% (w/w) amine fluoride[b] 0.08% (w/w) NaF 0.27% (w/w) $SnCl_2$	125 375	1400
Experiment 2				
Positive control (pH 4.2)[a]	250	0.16% (w/w) amine fluoride[b] 0.05% (w/w) SnF_2	125 125	409
Group 2.1	500	0.16% (w/w) amine fluoride[b] 0.08% (w/w) NaF 0.19% (w/w) $SnCl_2$	125 375	1000
Group 2.2	500	0.16% (w/w) amine fluoride[b] 0.08% (w/w) NaF 0.15% (w/w) $SnCl_2$	125 375	800
Group 2.3	250	0.16% (w/w) amine fluoride[b] 0.03% (w/w) NaF 0.19% (w/w) $SnCl_2$	125 125	1000
Group 2.4	250	0.16% (w/w) amine fluoride[b] 0.03% (w/w) NaF 0.15% (w/w) $SnCl_2$	125 125	800

[a] Meridol® mouth rinse.
[b] Olaflur, all mouth rinses were provided by GABA International AG, Therwil, Switzerland.

In cases of distinct substance loss, the reproducibility (10 repeated tracings of one sample) was ±0.8 μm and in cases of slight substance loss, the reproducibility was ±0.9 μm. The repeated analysis of one trace showed a standard deviation of ±0.1 μm. The variance of profiles of untreated specimens was 0.3 ± 0.5 μm.

2.4. Statistics

The statistical analysis of data was performed with SPSS 15.0 for Windows (SPSS, Chicago, IL, USA). The Kolmogorov–Smirnov test was used for checking the normal distribution of data. An analysis of variance (ANOVA) with Tukey's post hoc test was performed to compare the groups within one experiment. The level of significance was set at 0.05.

3. Results

In experiment 1, the tissue loss (mean ± SD) was highest in the control group (107.5 ± 7.9 μm). All mouth rinses reduced tissue loss significantly compared with the control group ($p \leq 0.001$). After application of the positive control solution, the tissue loss was 61.5 ± 9.1 μm. Within the three experimental mouth rinses, tissue loss increased with decreasing tin concentration. Values obtained for the 2800, 2100 and 1400 ppm mouth rinses were 21.1 ± 4.0 μm (group 1.1), 23.4 ± 4.0 μm (group 1.2) and 32.4 ± 6.0 μm (group 1.3), respectively.

In experiment 2, values for tissue loss were again highest in the control group (82.6 ± 18.7 μm). As in experiment 1, all mouth rinses significantly reduced tissue loss ($p \leq 0.001$). After treatment with the positive control solution, we measured tissue loss of 41.6 ± 13.0 μm. The application of both 500 ppm F^- mouth rinses led to comparable results (group 2.1: 1000 ppm Sn^{2+}: 26.9 ± 7.3 μm; group 2.2: 800 ppm Sn^{2+}: 21.6 ± 13.0 μm). After treatment with the 250 ppm F^-, 1000 ppm Sn^{2+} (group 2.3) and 250 ppm F^-, 800 ppm Sn^{2+} (group 2.4) mouth rinses, tissue loss was 24.6 ± 9.6 and 37.8 ± 5.8 μm, respectively.

The results, expressed as relative tissue loss (%), are displayed in Fig. 1.

4. Discussion

The present study was planned as a follow-up of several studies to investigate the efficacy of various tin-containing fluoride solutions in vitro[10,16] and in situ.[21] A relatively strong erosive protocol was chosen to simulate the situation in patients at high risk of dental erosion, like patients with eating

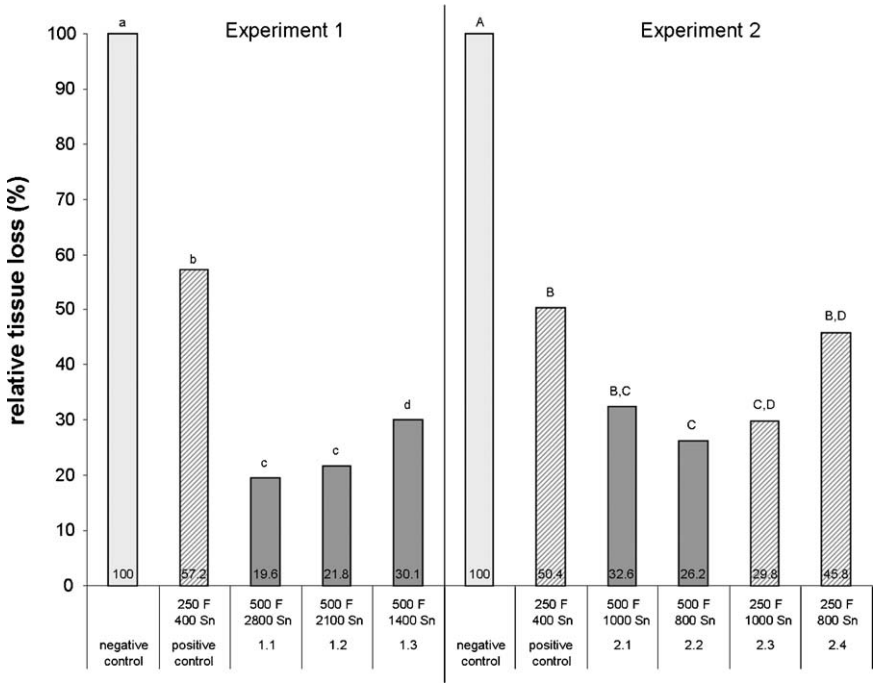

Fig. 1 – Relative tissue loss (%) in all groups of experiment 1 and experiment 2 after 10 days of cyclic demineralisation and remineralisation (6 × 5 min erosive demineralisation with 0.05 M citric acid and 2× 2 min treatment with experimental mouth rinses each day). Statistical significance of differences between groups is indicated by different indices (experiment 1 lowercase letters; experiment 2 uppercase letters; ANOVA with Tukey's post hoc).

disorders in combination with vomiting[22] or in persons living on a vegetarian diet.[23] To achieve comparability to the previous studies, the same protocol was used in the present investigation. The application of the experimental mouth rinses two times per day for two minutes appears relatively long, but the comparison of our in vitro and in situ studies showed that the in vitro results had good predictive value for in situ results, with clinically relevant application times of 1× 30 s per day. The commercially available AmF/SnF_2 mouth rinse showed good efficacy in previous studies, with a reduction of an erosive tissue loss in enamel by 60–90% in vitro[6,10] and 55% in situ (own results, unpublished). Therefore, this solution was suitable as positive control.

In both experiments, the highest substance loss was found in the control group; the reduction of mineral loss by the positive control solution ranged between 40 and 50%. These values were expected, based on previous studies.[6,10]

In the first experiment, the efficacy of the experimental mouth rinses increased with an increasing tin concentration. This was in concordance with a study by Wachtel,[24] which investigated the effect on enamel dissolution in a caries model by a single application of various stannous ion-containing fluoride solutions with various tin concentrations but a constant fluoride concentration. The study has shown a dose–response relationship between the tin concentration of the solution and the protection against enamel dissolution.[24] Comparing the results of the present study and an own previously performed study, using solutions with the same tin concentrations,[16] but two- or threefold higher fluoride concentrations, revealed that the percentage reduction of tissue loss was roughly the same in both studies and is independent of the fluoride concentration. Therefore, one can assume that with fluoride concentrations between 500 and 1500 ppm, the efficacy of F/Sn-solutions with a high concentration of tin (ranging between 1400 and 2800 ppm Sn^{2+}) predominantly depends on the concentration of the stannous ion rather than the fluoride ion.

The results of the second experiment are quite interesting. Amongst these groups, there are only vague indications of dose–response relationships. Regarding both 500 ppm F^- solutions (group 2.1, 1000 ppm Sn^{2+} and group 2.2, 800 ppm Sn^{2+}), there is no statistical difference. The same applies for the groups with 1000 ppm Sn^{2+} but different fluoride concentrations (group 2.1, 500 ppm F^- and group 2.3, 250 ppm F^-). On one hand, it could be that the differences in concentrations were too small to reveal differences in the efficacies of the various experimental mouth rinses. On the other hand, the role of the interaction between the fluoride and the stannous ions remains to be elucidated. However, it is clear that the presence of both ions is relevant for the efficacy. A study investigating the efficacy of various compounds as anti-erosive agents has shown that a pure sodium fluoride and a pure stannous chloride solution, had only moderate erosion-inhibiting effects, whereas a pure stannous fluoride solution nearly completely inhibited tissue loss.[6] The factors that are most relevant for the anti-erosive effect (e.g., the F-/Sn-ratio) are still unknown. In group 2.4 and the positive control of experiment 2, the total amount of fluoride was 250 ppm, and the total amount of tin was 400 and 800 ppm, respectively. Regarding the results of these groups, one can suppose that the efficacy also distinctly decreases when the concentrations are reduced below a minimum value. In this case, the total

formulation of a solution might become more relevant and could possibly have an impact on the availability of the ions. The experimental mouth rinses tested in the present study were ready-to-use formulations containing not only a mixture of the active agents, but also stabilisers and further additives. It is conceivable that these agents also have an impact on efficacy.

Within the limitations of an in vitro study, one can conclude from the results of this study that the concentration of the active agents can be distinctly reduced without sacrificing efficacy. This would avoid unnecessary exposure to high concentrations of active ingredients as well as the unpleasant, astringent feeling of highly concentrated solutions. The combination of 800 ppm Sn^{2+} and 500 ppm F^- seems to be a meaningful compromise between efficacy and mouth rinse acceptance.

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