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Protective effect of experimental mouthrinses containing NaF and TiF_4 on dentin erosive loss *in vitro*

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ABSTRACT

Objective: This *in vitro* study assessed the anti-erosive effect of experimental mouthrinses containing TiF_4 and NaF on dentin erosive loss. Material and Methods: Bovine dentin specimens were randomly allocated into the groups (n=15): 1) $SnCl_2$/NaF/AmF (Erosion Protection®/GABA, pH 4.5, positive control); 2) experimental solution with 0.0815% TiF_4 (pH 2.5); 3) 0.105% NaF (pH 4.5); 4) 0.042% NaF+0.049% TiF_4 (pH 4.4); 5) 0.063% NaF+0.036% TiF_4 (pH 4.5); 6) no treatment (negative control). Each specimen was cyclically demineralized (Sprite Zero, pH 2.6, 4x90 s/day) and exposed to artificial saliva between the erosive challenges for 7 days. The treatment with the fluoride solutions was done 2x60 s/day, immediately after the first and the last erosive challenges of the day. Dentin erosive loss was measured by profilometry (µm). The data were analyzed using Kruskal Wallis/ Dunn tests ($p<0.05$). Results: Mouthrinses containing TiF_4 or Sn/F were able to show some protective effect against dentin erosive loss compared to negative control. The best anti-erosive effect was found for experimental solution containing 0.0815% TiF_4 (100% reduction in dentin loss), followed by 0.042% NaF+0.049% TiF^4 (58.3%), $SnCl_2$/NaF/AmF (52%) and 0.063% NaF+0.036% TiF_4 (40%). NaF solution (13.3%) did not significantly differ from control. Conclusion: The daily application of experimental mouthrinse containing TiF_4 and NaF has the ability to reduce dentin erosion, as well as Erosion Protection® and TiF_4 alone.

Keywords: Dentin. Topical fluorides. Titanium. Tooth erosion.

INTRODUCTION

While the occurrence of dental caries has decreased during the last decades, researchers had focused on non-carious lesions, including erosion[10]. Dental erosion is an acid-induced tooth loss not involving microorganisms caused by external and/or internal acids[11]. Recent studies indicate a meaningful increase in dental erosion prevalence, especially in dentin, due to modifications in diet, lifestyle and socioeconomic status[1,17,24]. Early signs of erosive tooth loss have been found in children and young people[2,16].

Considering that the modification in population habits and the decrease of acid exposure are very tough, alternatives to reduce the progression of tooth erosive loss have been investigated. NaF is one of the most tested fluoride salt, whose mechanism of action against erosion is based on the deposition of CaF_2-like layer on the surface promoting an additional barrier that inhibits the contact of the acid with the tooth[5,7,15]. However, the anti-erosive effect of NaF on dentin is limited since its effect is only seen when the demineralized organic matrix (DOM) is preserved[5,18]. Nevertheless, some loss of the DOM by enzymatic activity is expected in the clinical situation, especially in patients with eating disorders[19].

Therefore, the use of other fluoride salts is likely to supply the lack of action of conventional fluoride (NaF) on dentin, and consequently, it could be more effective against erosion. Accordingly, titanium tetrafluoride (TiF_4) has been widely studied against tooth erosive demineralization since 1997[3], demonstrating the enamel erosion-inhibiting effect[8,9,12,25,28]. On the other hand, few studies have been performed on dentin. Generally, TiF_4 has a similar effect as NaF on the prevention of dentin erosion when it is applied as varnish[13,14], or a better effect than NaF when applied as high F concentrated solution[26,27]. Therefore, the protective effect of TiF_4 on dentin erosion is still in debate.

Considering that the application of professional fluoride, such as varnish, is not often done, patients at high risk of erosion would benefit from other alternatives to increase the frequency of fluoride exposure. Accordingly, the daily application of mouthrinses with low concentration of F, as those containing $SnCl_2$/AmF/NaF marketed in Europe (Erosion Protection®), has shown some protective effect against enamel and dentin erosion *in vitro* and *in situ*[6,20].

Therefore, the use of low concentrated TiF_4 mouthrinse by the patient could be a good alternative; however, there is a clinical limitation due to the low pH of the solution, which might cause some side effects in oral cavity, since it has cytotoxic effect on fibroblasts[21].

This study hypothesized that the formulation of an experimental solution containing both NaF and TiF_4 would increase the pH, allowing its use in the clinical situation without losing its protective effect against dentin erosion compared to TiF_4 alone and the commercial solution Erosion Protection®. The null hypothesis is that there is no significant difference in the protective effect against dentin erosive loss among the tested fluoride mouthrinses.

MATERIAL AND METHODS

Preparation of the dentin samples

Ninety dentin samples, which were stored in 0.1% thymol/0.9 % NaCl solution during the preparation phase, were cut from bovine dental roots. The root was separated from the crown using a water-cooled diamond saw and a cutting machine (IsoMet Low Speed Saw, Buehler, Lake Bluff, IL, USA), and embedded in Pre-30 self-polymerized acrylic resin in cylindrical shape to facilitate the handling. Thereafter, they were serially flattened with water-cooled abrasive discs (320, 600, and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA), and finally polished with felt paper wetted with a diamond solution (1 µm thickness of particles; Buehler, Lake Bluff, IL, USA) on a rotating polishing machine (Arotec SA Ind. e Com, Cotia, SP, Brazil). After polishing, the samples were cleaned in an ultrasonic device with deionized water for 2 min.

The reference areas on the polished dentin surface were marked with two parallel lines made with a scalpel blade, 1.0 mm apart. Small drilling was also done on the outer area of the dentin surface to allow the correct position of the sample in the profilometric system. Prior to the experiment, the baseline profile was measured and two layers of nail varnish (Colorama, Com. Ind. Exp Ltda., São Paulo, SP, Brazil) were applied on 2/3 of the control surface (sound surfaces), leaving only 1/3 central of the exposed dentin (1.0 mm x 5 mm).

Fluoride treatment

Dentin samples were randomly allocated to each of the six treatment groups (n=15): 1) commercial $SnCl_2$/NaF/AmF solution (800 ppm Sn^{+2}, 500 ppm F^-, pH 4.5, Erosion Protection®, GABA Int. AG, Basel, Switzerland, positive control); 2) experimental 0.0815% TiF_4 solution (315 ppm Ti^{+4}, 500 ppm F^-, pH 2.5); 3) experimental 0.105% NaF solution (500 ppm F^-, pH 4.5 adjusted with phosphoric acid); 4) experimental 0.042% NaF+0.049% TiF_4 solution (NaF- 190 ppm F^-, TiF_4 – 190 ppm Ti^{+4} and 300 ppm F^-, pH 4.4); 5) experimental 0.063% NaF+0.036% TiF_4 solution (NaF – 285 ppm F^-, TiF_4 – 140 ppm Ti^{+4} and 220 ppm F^-; pH 4.5); 6) no treatment (untreated, negative control). All solutions had approximately 500 ppm F^- based on the calculation obtained from the salts concentrations diluted in deionised water, and their pH was measured using a pH electrode. The experimental fluoride solutions were prepared using the analytical grade reagents from Sigma-Aldrich (St. Louis, MO, USA).

The fluoride treatments were performed twice a day (immediately after the first and the last erosive challenges of the day; v=0.5 ml/sample) for 1 min, during 7 days of erosive challenges. Excess of the solution was removed from the surface using a cotton roll.

Erosive challenges

Samples were submitted to a 7-day erosive de- and remineralization cycling. Erosive challenges took place by immersion in a freshly opened bottle of soft drink (Sprite Zero, Coca-Cola Company Spal, Porto Real, RJ, Brazil, pH 2.6, 30 ml/sample) four times a day for 90 s each, at 25°C. Then, the samples were rinsed with deionized water (5 s) and exposed to artificial saliva (pH 6.8, 30 ml/samples, 25°C) for 2h between the erosive challenges and overnight. The artificial saliva (v=500 ml) consisted of 0.001 g ascorbic acid, 0.015 g glucose, 0.290 g NaCl, 0.085 g $CaCl_2$, 0.080 g NH_4Cl, 0.635 g KCl, 0.080 g NaSCN, 0.165 g KH_2PO_4, 0.100 g carbamide and 0.170 g Na_2PO_4, and it was daily renewed [22].

Profile measurement

Dentin erosive loss (µm) was quantitatively determined by a contact profilometer (Mahr Perthometer, Göttingen, Lower Saxony, Germany)

Table 1- Median (minimum-maximum) of the dentin erosive loss for different groups

Solutions	Median (min; max)
Erosion Protection® (positive control)	0.86 (0.67; 1.85)[bc]
TiF_4 (0.0815%)	-0.19 (-0.45; -0.05)[a*]
NaF (0.105%)	1.56 (1.01; 2.38)[cd]
NaF+TiF_4 (0.042%+0.049%)	0.75 (0.21; 1.59)[ab]
NaF+TiF_4 (0.063%+0.036%)	1.08 (0.59; 1.66)[bc]
Negative control	1.80 (1.23; 4.94)[d]

* Negative value means increase of the surface (deposition)

Different letters show significant differences among the groups; min=minimum; max=maximum (p<0.0001)

before (baseline) and after 7 days of experiment. For the profilometric measurement, the nail varnish was carefully removed using a scalpel and acetone solution (1:1 water). Samples were maintained 100% wet during the measurement to avoid shrinkage of the DOM. Five profile measurements were performed at exactly the same sites as the baseline measurement, at intervals of 0.5 mm. To achieve this outcome, the dentin samples presented the identification marks (small drillings made with drill 1/4) and were inserted into a metal device, allowing the stylus to be accurately repositioned at each measurement. Baseline and final profiles were done and compared using the software MahrSurf CXR20 (Mahr, Göttingen, Lower Saxony, Germany). The scans were superposed and the average depth of the under curve area was calculated (µm)[12]. For a better understanding of the treatments effect, the prevention fraction (%) of each treatment was calculated by comparing the medians (each treatment versus negative control).

Statistical analysis

The software GraphPad InStat version 2.0 for Windows (GraphPad Software, La Jolla, CA, USA) was used for the statistical analysis. The assumptions of equality of variances and normal distribution of data were checked using the Bartlett and Kolmogorov-Smirnov tests, respectively. Once the homogeneity was not achieved, the data from dentin loss (µm) were analyzed using Kruskal-Wallis followed by Dunn's test. The level of significance was set at 5%.

RESULTS

All experimental mouthrinses promoted significantly lower dentin erosive loss when compared to the negative control (p<0.0001), except NaF solution (prevention fraction of 13.3%; p>0.05). The best anti-erosive effect was found for experimental solutions containing 0.0815% TiF_4 (prevention fraction of 100%) and 0.042% NaF+0.049% TiF_4 (58.3%). $SnCl_2$/NaF/AmF (Erosion Protection®, 52%) and 0.063% NaF+0.036% TiF_4 (40%) did not significantly differ from 0.042% NaF+0.049% TiF_4 and NaF alone, but both were less effective than TiF_4 alone. The median values (minimum-maximum) of dentin erosive loss for each group are shown in Table 1.

DISCUSSION

Considering the increase of dental erosion's prevalence[1,2,16,17,24], the attention has been focused on the development of preventive approaches to reduce the progression of this dental condition. The present study investigated the protective effect of the daily application of solution containing TiF_4/NaF. The null hypothesis tested in this study was rejected because the tested fluoride mouthrinses had a significantly different effect among them against dentin erosive loss. The solution containing pure TiF_4 showed the best protective effect, differing from all other groups except from a specific combination of TiF_4 and NaF.

This new approach would benefit patients with high risk of erosion presenting gingival recession due to periodontal disease, brushing habits (abrasion) or/and occlusal disorders (abfraction). The dentin, in these cases, may be likely exposed to extrinsic acid sources from the diet, and therefore, susceptible to the development of erosion.

The present study aimed to simulate the home-care application of low-concentrated fluoride (500 ppm F^-) solution, after two meals (morning and evening), in periods in which the patient could perform a rinse after the daily hygiene habit. The idea behind the combination of two fluorides, TiF_4 and NaF, into an experimental mouthrinse is based on the fact that pure TiF_4 has low pH, impairing its clinical use. The addition of NaF to TiF_4 solution was able to increase its pH to a suitable value to be applied *in vivo* and to be compared with commercial products.

In this study, TiF_4 alone reduced in 100% dentin loss, which might be due to the deposition of acid-resistant surface layer rich in CaF_2, titanium dioxide and hydrated titanium phosphate (unpublished data). The protocol of TiF_4 application tested in this study has not been applied in previous studies, since most of them tested high concentrated TiF_4 solution applied at once[8,9,12-14,25-28]. The addition of NaF into TiF_4 solution decreased the protective effect, considering the percentage of prevention fraction, due to a likely lower precipitation of Ti and F salts. However, one of the combinations (0.042% NaF+0.049% TiF_4) was still statistically similar to pure TiF_4 solution.

The present results in respect to the daily application of fluoride mouthrinses are more promising than those found for a unique application of a product with high concentration of fluoride, as varnish, against dentin erosion and erosion-abrasion[13,14]. The findings suggest the importance of a frequent low concentrated fluoride exposure rather than a unique application of a high concentrated fluoride product.

A recent study was conducted in enamel showing similar results[22]. However, only one of the combinations (0.042% NaF+0.049% TiF_4) was effective in reducing enamel erosive loss (41% preventive fraction), while the other one did not differ from the negative control. Generally, the tested fluoride mouthrinses had better impact on dentin compared to enamel, which might be explained by the differences in the composition between the dental tissues. In case of dentin, the effect of the combinations of TiF_4 and NaF was similar to those provided by a commercial fluoride solution (positive control), which has been widely used in Europe for prevention of tooth erosion. The preventive fraction found by the application of Erosion Protection® in the present study was similar to a previous *in situ* study performed by other research group[6]. Based on this finding, we can speculate that the acid-resistance of Ti and F precipitates found for our experimental solutions are similar to tin and fluoride precipitates produced by the application of Erosion Protection® on dentin.

On the other hand, NaF solution presented the worst performance, not differing from the negative control. It is widely known that NaF is ineffective to protect against tooth erosion[9,12,15,23,28] especially in case of dentin, in which its effect depends on the presence of the DOM[5,18]. In this study, the DOM was not removed, but it would be interesting to test the effect of the experimental fluoride solutions on dentin without DOM. Further studies should also test the effect of the experimental fluoride solutions on both dentin erosion and brushing abrasion to check the stability of the protective effect faced by two different challenges (chemical and mechanical).

Another point to consider is that erosion in dentin is very complex due to the role of DOM in the progression of erosive loss[18]. Therefore, erosive loss is difficult to be quantified, since the quality of the remaining organic layer may interfere with the profilometric measurement. Shrinkage of the DOM may occur under different environments interfering in the profile analysis. Therefore, to generate reliable data, the profiles must be measured with the samples immersed 100% in water or without DOM. We have decided to perform the analysis with DOM under 100% humidity, since in previous study we have not found differences in the comparison between TiF_4 and NaF varnishes in the profile analysis of dentin with or without DOM[4].

The present study showed promising results for the experimental fluoride mouthrinses. Future studies, including *in situ* and *in vivo* models, must be performed to confirm the findings, since saliva can be able to buffer the pH of the fluoride solutions, which might lead to different results.

CONCLUSION

Under the conditions of the present study, we can conclude that the daily application of an experimental mouthrinse containing a specific combination of TiF_4 and NaF has the ability to reduce dentin erosion *in vitro*, and may be a good alternative for high-risk populations.

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