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Original

Effect of xylitol and fluoride on enamel erosion *in vitro*

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Abstract: This study aimed to determine the anti-erosive effects of xylitol, fluoride and a xylitol/fluoride combination used as an additive in an acidic drink or as mouthrinse after enamel was exposed to an acidic drink, *in vitro*. Human third molars were divided into 7 groups (A-G). Samples from groups A to D were immersed for 5 min in orange juice only (A), orange juice plus either 25% xylitol (B), F^- 1 ppm (C) or a 25% xylitol/F^- 1 ppm combination (D), respectively. Samples from groups E to G were immersed in orange juice for 5 min and then in either 40% xylitol (E), F^- 227 ppm (F) or a 40% xylitol/F^- 227 ppm combination (G), for 1 min respectively. This process was performed four times daily for 14 days. Mineral loss was determined from the lesion depth and surface hardness. Erosion depth progressively increased in all groups, except E, where erosion depth was significantly lower than group A. Surface microhardness progressively decreased in all groups, except E, where hardness was significantly higher than group A. This study demonstrated that addition of xylitol, fluoride or a xylitol/fluoride combination to an acidic drink or post-treatment with fluoride or a xylitol/fluoride combination could reduce, but not prevent, enamel erosion. (J. Oral Sci. 49, 293-297, 2007)

Keywords: xylitol; fluoride; enamel erosion; *in vitro*.

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Introduction

Dental erosion is defined as chronic localized tooth surface loss caused by non-bacterial acid dissolution. Erosive tooth loss is an ongoing problem that can occur at an early age and is currently believed to be the most common mode of tooth wear (1,2). The etiology of dental erosion may be either extrinsic or intrinsic in origin. Dietary acids including acidic drinks and beverages are the most common extrinsic cause (3,4). Many attempts have been made to modify the composition of drinks and reduce their erosive potential by adding calcium citrate malate or mixtures supplying calcium and phosphate salts (5-8). However, these modifications may affect the flavor and pH of the drinks depending on the salt and the concentration used (5).

In addition to its anti-cariogenic properties, fluoride has also been demonstrated to have an anti-erosive potential (9-12). Sorvari et al. showed that the addition of 15 ppm fluoride in sports drink could reduce the severity of dental erosion in rats (13). Additionally, in an *in vitro* experiment, treatment of human enamel with topical fluoride, 2.26% Duraphat varnish or 1.2% NaF solution, for 48 h before exposure to an acidic beverage could inhibit initial erosion (14). These findings were not supported by the results of Larsen and Richards who demonstrated that fluoride, neither in the form of calcium fluoride on the enamel nor as a part of the drink itself, was able to provide a preventive effect against dental erosion (15).

Xylitol is a non-sugar sweetener that has been permitted for use in foods for many years. It can be found naturally in small quantities in fruits including berries and vegetables (16). Xylitol is claimed to have both cariostatic and anti-

cariogenic effects (17). Xylitol in high concentration is known to form complexes with Ca^{2+} (18), penetrate into demineralized enamel, and interfere with the transport of dissolved ions from the lesion to the demineralizing solution (19). Recently, Amaechi et al. reported that xylitol and fluoride have an additive effect in the reduction of bovine dental erosion when added to pure orange juice (20). The aim of this *in vitro* study was to investigate the anti-erosive effect of xylitol, fluoride and a xylitol/fluoride combination used as an additive in pure orange juice or as a mouthrinse after human dental enamel was exposed to an acidic drink.

Materials and Methods

Preparation of enamel specimens

Eighty-four extracted, caries-free, human third molars were obtained from the Dental Hospital, Faculty of Dentistry, Prince of Songkla University, Songkhla, Thailand. The teeth were kept in 0.1% thymol solution until use. Crowns were sectioned from the roots, rinsed with water and examined under a stereomicroscope for any observable defects. Only intact enamel areas on the buccal surface were used in this study. The specimens were embedded in acrylic resin (Meliodent®, Bayer Dental, Leverkusen, Germany) in planar parallel moulds and lightly ground on a rotating and polishing machine (Jean Wirtz Phoenix 4000, Jean Wirtz GmbH & Co KG, Dusseldorf, Germany) with silicon carbide papers of grain size 600, 1,000, and 1,200 grit under constant tap water cooling, to produce a flat surface with no more than an average of ± 0.3 μm deflection (Ra) on a baseline profile using a profilometer (Surfcorder SE-2300, Kosaka Laboratory Ltd., Tokyo, Japan). A carbon pencil was used to map out a horizontal rectangular area (1.5 × 1.5 mm) in the middle of the enamel surface of each tooth for an exposed window. The outside areas of the mapped window were coated with two layers of acid resistant nail varnish. All specimens were then stored in a normal saline solution for later use.

Erosion experimental design

Pure commercially available orange juice in a wax paper package, assumed to contain no additives, was purchased (Malee, batch no. K11, Bangkok, Thailand). The pH of the orange juice was measured with an Orion pH meter (Orion, Boston, MA, USA). The mean pH of the orange juice was 3.26 ± 0.04. The tooth specimens were randomly divided into seven groups (groups A-G). Samples from groups A to D were immersed for 5 min in pure orange juice only (group A), pure orange juice supplemented with either 25% (w/v) xylitol (group B), 0.00022% NaF (equivalent to 1 ppm F; group C), and a 25% (w/v) xylitol / 1 ppm F combination (group D), respectively. Samples from groups E to G were immersed first in pure orange juice for 5 min and then immersed in a solution containing either 40% (w/v) xylitol (group E), 0.05% NaF (equivalent to 227 ppm F; group F), or a 40% (w/v) xylitol / 227 ppm F combination (group G), for 1 min respectively. The concentration of xylitol and fluoride added to the drink was chosen so that it would not affect the flavor of the drink or increase the risk of over consumption (20). The concentration of xylitol and fluoride used as a post-treatment solution was based on the concentration generally used in mouthrinse.

The specimens were immersed in a continuously stirred uniform volume (20 ml/specimen) of their assigned agent at regular intervals four times daily for a total of 14 days. The immersion was done at room temperature, approximately 25°C. Between exposures and during 12 h overnight, the samples were stored in artificial saliva (21). The erosive agents and artificial saliva were changed daily. The pH of the erosive agents was measured and no change was observed from that of the original orange juice. At the end of the cycling period, the specimens were washed with deionized water and blot-dried. Profilometry and surface microhardness were evaluated before immersion (day 0) and after exposure to erosion on days 1, 3, 7, and 14.

Surface microhardness measurement

A microhardness tester (Buehler Micromet II, Buehler Ltd., Lake Bluff, IL, USA) with a Vickers diamond head under a load of 200 g was used to determine possible changes in surface microhardness during the experiment. The measurement was made initially and after exposure to erosion on days 1, 3, 7, and 14. Five well-formed indentations on each specimen were measured to calculate the mean Vickers hardness value for each test.

Profilometry measurements

Erosion depth (expressed in μm) was determined profilometrically with a Surfcorder SE-2300® (Kosaka Laboratory Ltd.) equipped with a mechanical pick-up. The erosion depth of the polished samples was calculated from the profile depth, defined as the vertical distance between the highest and lowest point of a given profile tracing. Three tracings were made on each specimen. The maximum erosive depth (maximum peak to valley height, Rmax) was recorded and averaged out.

Statistical analysis

Analyses of variance and covariance with repeated measurement were used to study the effects of assigned

regimens, immersion time on the erosion depth and surface microhardness both within and between groups. The Tukey HSD multiple comparison was used to compare the difference between groups. In all tests, the level of significance was set as $P = 0.05$.

Results

The mean baseline erosion depth (Rmax) of samples in each group before immersion in assigned erosive reagents (day 0) ranged from 0.96 ± 0.16 μm to 1.38 ± 0.23 μm. The erosion depth progressively increased over time in all groups and significantly increased ($P < 0.05$), when compared with the baseline of the same group (Table 1). When comparing the difference of the erosion depth between groups after 14 days of treatment using Tukey HSD multiple comparison, we found no significant difference between group A and E ($P = 0.520$, Table 1, i), group F and G ($P = 0.641$, Table 1, ii), and group B and D ($P = 0.218$, Table 1, iv), but group C (iii) was significantly different from all groups ($P < 0.05$). Erosion depth could be ranked and grouped from the highest to lowest as follows: A~E > F~G > C > B~D ($P < 0.05$).

The mean initial enamel hardness of the specimens ranged from 308.21 ± 4.92 to 320.96 ± 5.50 Vickers units when measured at the baseline. The surface hardness progressively decreased over time and was significantly decreased ($P < 0.05$), when compared with the baseline in all groups (Table 2). When comparing the difference of the surface hardness between groups after 14 days of treatment using Tukey HSD multiple comparison, we found no difference between group A and E ($P = 0.949$, Table 2, i), group F and G ($P = 0.724$, Table 2, ii), and group B and D ($P = 0.303$, Table 2, iv), but group C (iii) was significantly different from all groups ($P < 0.05$). Surface hardness could be ranked and grouped from the highest to lowest as follows: B~D > C > F~G > A~E ($P < 0.05$).

Table 1 Mean erosion depth (Rmax) of enamel after various treatments (n = 6) at day 0, 1, 3, 7, and 14

Group	Rmax (μm ± SD)				
	Day 0	Day 1	Day 3	Day 7	Day 14
A	1.11 ± 0.09	3.73 ± 0.49	8.49 ± 0.89	16.25 ± 1.43	27.19 ± 1.66^{i}
B	1.16 ± 0.25	1.66 ± 0.38	3.69 ± 0.60	7.47 ± 1.17	12.46 ± 1.84^{iv}
C	1.12 ± 0.23	1.98 ± 0.45	5.39 ± 0.97	9.83 ± 1.14	16.94 ± 1.65^{iii}
D	0.96 ± 0.16	1.24 ± 0.80	3.27 ± 0.53	6.15 ± 0.67	9.57 ± 0.99^{iv}
E	1.31 ± 0.28	2.93 ± 0.52	8.50 ± 1.19	14.74 ± 1.38	25.25 ± 2.45^{i}
F	1.28 ± 0.35	3.07 ± 0.63	7.64 ± 0.70	12.76 ± 1.02	21.03 ± 1.17^{ii}
G	1.38 ± 0.23	2.91 ± 0.27	7.45 ± 0.79	12.15 ± 1.69	18.29 ± 1.77^{ii}

A: pure orange juice only; B: pure orange juice supplemented with 25% xylitol; C: pure orange juice supplemented with 1 ppm F; D: pure orange juice supplemented with a 25% xylitol / 1 ppm F combination; E: post-treatment with 40% xylitol; F: post-treatment with 227 ppm F; G: post-treatment with a 40% xylitol / 227 ppm F combination.
Statistically significant difference between i, ii, iii and iv ($P < 0.05$)

Table 2 Mean surface microhardness (Vickers hardness) of enamel after various treatments (n = 6) at day 0, 1, 3, 7, and 14

Group	Surface microhardness (kg/mm^2 ± SD)				
	Day 0	Day 1	Day 3	Day 7	Day 14
A	314.82 ± 3.78	219.02 ± 8.51	179.24 ± 9.35	97.01 ± 6.33	43.15 ± 2.28^{i}
B	318.37 ± 12.94	244.03 ± 8.22	206.88 ± 9.60	169.02 ± 6.06	108.07 ± 5.05^{iv}
C	315.74 ± 4.44	246.36 ± 12.86	205.19 ± 8.87	137.72 ± 4.13	87.82 ± 3.24^{iii}
D	315.23 ± 4.39	268.30 ± 6.36	211.21 ± 9.07	175.53 ± 5.48	111.30 ± 6.74^{iv}
E	320.96 ± 5.50	217.87 ± 10.29	179.68 ± 10.38	101.01 ± 7.54	49.54 ± 7.80^{i}
F	308.21 ± 4.92	221.78 ± 10.97	192.28 ± 10.60	118.78 ± 8.11	78.28 ± 9.84^{ii}
G	311.06 ± 10.83	225.55 ± 12.57	202.29 ± 12.65	122.46 ± 10.43	82.07 ± 9.71^{ii}

A: pure orange juice only; B: pure orange juice supplemented with 25% xylitol; C: pure orange juice supplemented with 1 ppm F; D: pure orange juice supplemented with a 25% xylitol / 1 ppm F combination; E: post-treatment with 40% xylitol; F: post-treatment with 227 ppm F; G: post-treatment with a 40% xylitol / 227 ppm F combination.
Statistically significant difference between i, ii, iii and iv ($P < 0.05$)

Discussion

The present study aimed to determine the *in vitro* anti-erosive effect of xylitol, fluoride and a xylitol/fluoride combination used as an additive in an acidic drink or as a rinse during repeated acid challenge. Surface microhardness measurement and profilometric analysis were used to get information on enamel softening and tooth mineral loss in connection with the acid-induced demineralization. These two recommended measurements have been used to study enamel softening after immersion in acidic drink by several investigators (9,14). Our results clearly showed that post-treatment with 40% xylitol alone, after being exposed to an acidic drink, was unable to reduce tooth surface and mineral loss. The addition of xylitol, fluoride, and a xylitol/fluoride combination to the acidic drink or post-treatment with a solution containing either fluoride or a xylitol/fluoride combination after the acid challenge could reduce enamel dissolution, *in vitro*. However, the strongest anti-erosive treatment was to add xylitol or xylitol/fluoride combined to the drink itself.

When samples were immersed in the pure orange juice containing xylitol, high content of calcium in the pure orange juice and in the artificial saliva and the calcium released from the tooth surface during the onset of erosion would have facilitated the anti-demineralization activity of xylitol. Xylitol is able to form complexes with calcium ion (18) and prevent decalcification by inhibiting the translocation of dissolved Ca^{2+} and PO_4^{3-} ions from lesions (by lowering the diffusion coefficients of calcium and phosphate ions) (22). However, if the samples were immersed first in the demineralizing solution and then rinsed with xylitol solution, these mechanisms would have unlikely occurred. This might explain why application of xylitol after acid challenge as a rinse could not help to reduce enamel erosion, *in vitro*.

Fluoride ions in the solution are known to have a protective effect against tooth demineralization caused by caries and erosion (9,11,12,23). Hydroxyapatite might be dissolved below the critical pH (for hydroxyapatite), but the released mineral ions could be re-precipitated as fluoroapatite or a mixed fluorhydroxyapatite in the presence of a low concentration of fluoride in the solution (23). In the presence of high fluoride concentration (> 100 ppm F), calcium fluoride is formed and acts as a fluoride reservoir on the tooth surface. When enamel is subjected to a pH-cycling regime with fluoride present, the remineralization process occurs (23). In this study, the mechanism of reducing tooth demineralization by post-treatment with fluoride solution (227 ppm F) after the acid challenge might be explained by the CaF_2- like layer that persisted on the tooth surface and served as a reservoir of fluoride inducing the reprecipitation of minerals in the form of fluorapatite or fluohydroxyapatite, preventing loss of mineral ions.

Based on the data from Amaechi et al., xylitol and fluoride have an additive effect in inhibiting dental erosion (bovine incisors) *in vitro* when supplemented into orange juice (20). This conclusion seems to disagree with the results of our study. We found no significant difference in tooth surface hardness or erosion depth (human third molars) between the groups treated with orange juice supplemented with either xylitol alone or a xylitol/fluoride combination. These may be due to the differences in enamel types, temperature and exposure time used as suggested by Amaechi et al. (24). In that study, Amaechi et al. demonstrated that mineral loss and erosion depth of the enamel significantly increased with a rise in the exposure time and the temperature of the orange juice. In addition, they found that bovine permanent enamel eroded faster than human permanent enamel (24).

Our results demonstrated that addition of 1 ppm F to the drink provided a better inhibitory effect against dental erosion than post-treatment with 227 ppm F solution. However, we found that fluoride, neither in the form of a rinse nor as part of a drink, was able to provide a preventive effect against erosion, *in vitro*. Our conclusion seems to agree with the results of Larsen and Richards (15). The results of our study emphasized that the treatment listed above could not totally prevent erosion. Compared to the initial hardness and profile depth, distinct enamel softening and tooth mineral loss were observed in all groups. The acid-induced tooth mineral loss in erosion is much stronger than that caused by the caries process. That means that the anti-demineralization mechanisms of fluoride or xylitol are unlikely to function as efficiently as in the caries process, when teeth are literally immersed in acidic drink.

In conclusion, we demonstrated that xylitol, fluoride or xylitol/fluoride combined, either in the form of supplementation in a drink or in the form of a rinsing solution, could not prevent, but could reduce, demineralization caused by acidic drink. Addition of xylitol alone or in combination with fluoride to a drink is the most effective way to reduce dental erosion compared to other applications. Post-treatment with xylitol solution after the acid challenge was unable to reduce tooth demineralization.

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