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Effect of Low Fluoride Acidic Dentifrices on Dental Remineralization

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This study evaluated the capacity of fluoride acidic dentifrices (pH 4.5) to promote enamel remineralization using a pH cycling model, comparing them with a standard dentifrice (1,100 µgF/g). Enamel blocks had their surface polished and surface hardness determined (SH). Next, they were submitted to subsurface enamel demineralization and to post-demineralization surface hardness analysis. The blocks were divided into 6 experimental groups (n=10): placebo (without F, pH 4.5, negative control), 275, 412, 550, 1,100 µgF/g and a standard dentifrice (positive control). The blocks were submitted to pH cycling for 6 days and treatment with dentifrice slurries twice a day. After pH cycling, surface and cross-sectional hardness were assessed to obtain the percentage of surface hardness recovery (%SH_R) and the integrated loss of subsurface hardness (ΔKHN). The results showed that %SH_R was similar among acidic dentifrices with 412, 550, 1,100 µgF/g and to the positive control (Tukey's test; $p>0.05$). For ΔKHN, the acidic dentifrice with 550 µg F/g showed a better performance when compared with the positive control. It can be concluded that acidic dentifrice 550 µgF/g had similar remineralization capacity to that of positive control.

Key Words: dentifrices, dental enamel, fluoride, dental caries, tooth remineralization.

Introduction

As a result of the widespread availability of various fluoride products, dental caries rates have greatly reduced. However, this wide availability has also promoted an increase in dental fluorosis (1). Fluoride (F) dentifrices contribute to approximately 57% of the total fluoride ingestion by children aged 4 to 6 years because the swallowing reflex is not totally developed by children at this age (2). Therefore, some authors have emphasized the need for preventive measures to avoid excessive fluoride ingestion from dentifrices such as reducing the amount of fluoride placed on toothbrushes, limiting to twice a day the use of dentifrices during toothbrushing, supervising children during tooth brushing and developing dentifrices with low fluoride concentration (3).

However, fluoride reduction in dentifrices must be followed by the addition of sources capable to maintain a similar effectiveness to that of a standard dentifrice with 1,100 µgF/g. It is known that the main product formed after fluoride topical application is calcium fluoride (CaF_2), which is responsible for fluoride anticariogenic action. CaF_2 is a reservoir of fluoride and calcium and both are important ions to promote enamel remineralization (4). The higher CaF_2 formation, the greater fluoride availability during cariogenic challenge. Saxegaard and Rølla (5) showed that CaF_2 formation on enamel increases in acidic environments. Therefore, the caries prevention ability of low F dentifrices could be maintained by reducing its pH from the conventional neutral (7.0) to acidic (5.5 or lower).

Low F acidic dentifrices have shown to be able to interfere in enamel demineralization (6,7). A dentifrice with 550 µgF/g pH 5.5 showed the same ability in preventing enamel demineralization as a dentifrice with 1,100 µgF/g (6). Alves et al. (8) observed better results in dentifrices with 412 µgF/g pH 4.5. However, there is no data related to the capacity of these dentifrices to remineralize early caries lesions. A sensible methodology to verify dose response relationship in acidic dentifrices using an *in vitro* model (9) is required. Thus, the aim of this study was to evaluate the capacity of low fluoride acidic dentifrices (pH 4.5) in promoting enamel remineralization when compared with a standard dentifrice using an *in vitro* pH cycling model.

Material and Methods

Experimental Design

Enamel blocks (4 mm x 4 mm) obtained from bovine incisors had their enamel surfaces polished and surface hardness (SH) determined. After subsurface enamel demineralization, the blocks were submitted to post-demineralization surface hardness (SH_1) assessment and were randomized in six groups (n=10) according to mean percentage of mineral loss (-71.2% to -96.0%) and their confidence interval ($p<0.05$). Experimental acidic dentifrices were placebo (0 µgF/g), 275, 412, 550 and 1,100 µgF/g, pH 4.5. A commercial dentifrice was used as gold standard (Crest™, 1,100 µgF/g, pH 7.0). The enamel blocks were submitted to pH cycling for six days. Twice a day, the blocks were treated with dentifrice slurries. After pH cycling, surface (SH_2) and cross-sectional hardness were assessed to calculate, respectively, the percentage

of surface hardness recovery (%SH_R) and integrated loss of subsurface hardness (ΔKHN).

Toothpaste Formulation and Fluoride, pH and Phosphorus Assessment

The experimental dentifrices were manufactured by FGM Produtos Odontológicos Ltda and had the following ingredients: carboxymethylcellulose, sodium methyl-p-hydroxybenzoate, sodium saccharin, peppermint oil, glycerol, hydrated silica, sodium lauryl sulfate, water and sodium fluoride (NaF). NaF was added to achieve the desired concentration (275, 412, 550 and 1,100 µgF/g). The pH was set to 4.5 using phosphoric acid. A dentifrice without F and pH 4.5 (negative control) and a standard dentifrice (Crest; Procter & Gamble, Cincinnati, OH, USA, pH 7.0, 1,100 µg F/g, positive control) were also used.

Fluoride assessment in toothpastes was done according to Brighenti et al. (6). After water dispersion, a sample from the suspension was treated with 2 $M.L^{-1}$ HCl for total F assessment. For ionic F assessment, supernatants were obtained by centrifuge (906×g; 20 min). The same volume of TISAB II ("Total ionic strength adjustment buffer"; Orion Research Inc., Beverly, MA, USA) was added to the solutions. Fluoride measurements were performed with an ion-selective electrode Orion 96-09 (Orion Research Inc.) and an ion analyzer Orion 720 A+ (Orion Research Inc.) calibrated with standards containing 0.125 up to 4.0 µgF/mL. Phosphorus in toothpastes was measured according to the colorimetric determination as described by Fiske and Subbarow (10) in the supernatants obtained after centrifuge (906×g; 20 min).

The pH in dentifrices slurries (1:3 w/w) was determined using a pH electrode (2A09E; Analyser, São Paulo, SP, Brazil) calibrated with pH 7.0 and 4.0 standards.

Subsurface Enamel Demineralization

Before induction of subsurface enamel demineralization, enamel blocks were selected by surface hardness (SH) utilizing a microhardness tester (HMV-2000; Shimadzu Corp., Kyoto, Japan) attached to CAMS-WIN Software (NewAge Industries, Southampton, PA, USA) to analyze the images. Five indentations spaced 100 µm from each other were made at the center of the enamel surface (SH) (25 g, 10 s). Blocks with hardness values between 336.0 to 391.6 kgf/mm^2 were selected. Subsurface enamel demineralization was carried out using a modified model according to Queiroz et al. (11). The blocks were immersed individually in 32 mL of a solution containing 1.3 $mM.L^{-1}$ calcium, 0.78 $mM.L^{-1}$ phosphate in 0.05 $mM.L^{-1}$ acetate buffer, pH 5.0; 0.03 µg/mL F; for 16 h at 37°C (12). After that, post-demineralization surface hardness (SH_1) was measured with the same parameters described previously. Indentations for SH_1 were made 100 µm from each other and from the baseline indentations (SH). The percentage of surface hardness loss was calculated ([[SH1 - SH]/SH]*100) to randomize the enamel blocks in the treatment groups.

pH Cycling and Dentifrice Treatments

To evaluate the effect of dentifrice treatment on enamel remineralization, a pH cycling model based on Vieira et al. (9) was used. During six days the blocks were submitted to pH cycling at 37°C. The blocks were immersed individually in a remineralization solution (1.5 $mM.L^{-1}$ calcium, 0.9 $mM.L^{-1}$ phosphate, 150 $mM.L^{-1}$ potassium chloride in 0.02 $M.L^{-1}$ cacodylic buffer, pH 7.0; 0.02 µgF/mL, 1 mL/mm^2) for 22 h. The cariogenic challenge was promoted by a demineralization solution (2.0 $mM.L^{-1}$ calcium and phosphate in 75 $mM.L^{-1}$ acetate buffer, pH 4.7; 0.03 µgF/mL, 3 mL/mm^2) for 2 h *per* day. The solution was refreshed daily. Twice a day, enamel blocks were treated with toothpaste/deionized water slurries (1:3 w/w, 2 mL/block) under agitation (1 min). Deionized water rinses were performed between each step.

Hardness Analysis

After pH cycling, enamel surface hardness (SH_2) was determined using the same parameters above. Five indentations spaced 100 µm from each other and from the baseline indentations were performed. The percentage of surface hardness recovery (%SH_R = ((SH_2 - SH_1) / (SH - SH_1)) x100) was calculated (9).

Next, the enamel blocks were longitudinally sectioned through their center and embedded in acrylic resin with the cut face exposed and gradually polished. For the cross-sectional hardness measurements, three rows of nine indentations spaced 100 µm from each other were made at different distances from the outer enamel surface (10, 30, 50, 70, 90, 110, 130, 220 and 330 µm), under a 25 g load for 10 s. The mean value of each distance was calculated. Integrated hardness (KHN x µm) of the lesion into sound enamel was calculated by the trapezoidal rule (GraphPad Prism, version 3.02; GraphPad Software Inc., La Jolla, CA, USA) and subtracted from the integrated hardness of sound enamel to obtain the integrated loss of subsurface hardness (ΔKHN) (13).

Statistical Analysis

The analysis was performed by GraphPad Prism (version 3.02) software (GraphPad Software, Inc., SAn Diego, CA, USA), with a significance level of 5%. SH, SH_1, SH_2, %SH_R and ΔKHN were submitted to normality (Kolmogorov-Smirnov's) and homogeneity (Bartlett's) tests. The values for SH, SH_1, SH_2 and %SH_R were normal and homogeneous and were thus submitted to one-way analysis of variance and Tukey's

test. The values for ΔKHN were heterogeneous and were submitted to Kruskal-Wallis test followed by Dunn test. The hardness values in function of depth were submitted to two-way analysis of variance and Student-Newman-Keuls's test. Pearson's correlation coefficients were calculated considering fluoride and phosphorus concentration in dentifrice and also %SH and ΔKHN.

Results

The mean (SD) pH of dentifrice slurries during the treatments were 4.32 (0.27) for the acidified dentifrices and 7.56 (0.22) for the positive control group. Total and ionic fluoride and phosphorus in experimental dentifrices and positive control are represented in Figure 1. All dentifrices showed expected values for fluoride concentration. The acidified dentifrice with 1,100 µgF/g and positive control showed higher phosphorus concentration in comparison to other groups (Fig. 1).

The mean values of initial surface (SH) and post demineralization surface (SH_1) hardness were similar (p>0.05) among groups (Table 1). Hardness after pH cycling (SH_2), showed no statistically significant difference between groups 275 412, 550 and 1,100 when compared with positive control (p>0.05). Acidic dentifrices with 412, 550 and 1,100 µgF/g showed similar $\%SH_R$ to that of positive control (p>0.05) (Table 1). A good and positive correlation was observed between fluoride concentration in dentifrice and SH_2 (r=0.81; p<0.001) and between fluoride concentration in dentifrice and $\%SH_R$ (r=0.80; p<0.001). There was a mild positive correlation between P concentration in dentifrice and SH_2 (r=0.54; p<0.001), and $\%SH_R$ (r=0.56; p<0.001).

Regarding ΔKHN (Table 1), acidic dentifrice with 550 µgF/g showed lower values than positive control (p<0.05), but was similar to 412 and 1,100 µgF/g (p>0.05) and there were no differences between 412 µg F/g dentifrice and the positive control (p>0.05). A negative correlation was observed between ΔKHN and fluoride in dentifrice (r=-0.67; p<0.001) and between ΔKHN and phosphorus in dentifrice (r=-0.35; p=0.037). Figure 2 shows the cross-sectional hardness profiles at different depths in enamel blocks.

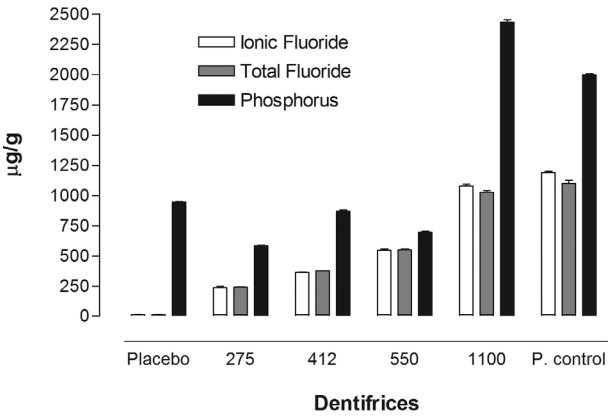

Figure 1. Graphic presentation of mean values of fluoride and phosphorus in the experimental dentifrices and positive control (n=6). The bars denote standard deviations.

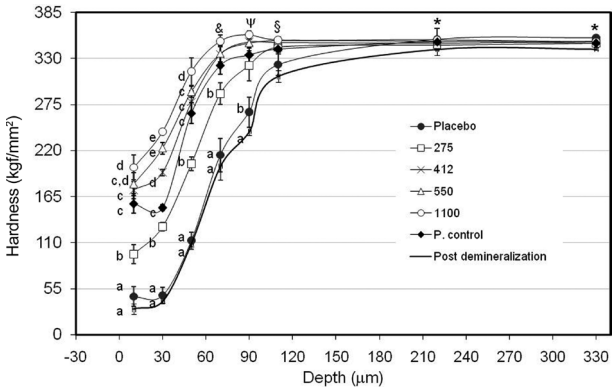

Figure 2. Cross-sectional hardness profiles (mean, n=10) at different depths in enamel blocks treated according to the experimental dentifrices and positive control. The bars denote standard deviations. Distinct letters represent statistically significant differences among groups in each depth (Student-Newman-Keuls; p<0.05). (&) not statistically different among groups 412, 550, 1100 and P. control. (ψ) not statistically different among groups 275, 412, 550, 1100 and P. control. (§) not statistically different among groups Placebo, 275, 412, 550, 1100 and P. control.

Table 1. Mean values (standard deviation) of hardness analysis (n=10) according to the different treatments

Dentifrice	SH	SH_1	SH_2	$\%SH_R$	ΔKHN
Placebo (no F)	370.2[a] (12.6)	53.0[a] (22.8)	73.8[a] (37.4)	6.8[a] (8.6)	18,316.7[a] (2,654.7)
275 µgF/g (experimental)	368.7[a] (17.5)	56.7[a] (28.8)	146.5[b] (35.3)	29.5[b] (7.2)	11,100.5[b] (2,228.0)
412 µgF/g (experimental)	372.0[a] (15.2)	55.0[a] (24.6)	164.5[b,c] (32.3)	34.8[b,c] (5.8)	6,152.3[c,e] (1,339.0)
550 µgF/g (experimental)	371.9[a] (12.5)	55.5[a] (20.3)	188.7[b,c] (43.1)	42.6[c] (11.2)	4,889.2[c,d] (1,024.9)
1,100 µgF/g (experimental)	365.2[a] (17.1)	48.8[a] (27.1)	205.8[c] (46.0)	50.4[c] (12.7)	4,247.5[c] (1,519.1)
Crest™ (1,100 µgF/g)	366.2[a] (16.5)	51.1[a] (23.8)	180.2[b,c] (37.8)	41.5[c] (8.9)	7,408.0[e] (857.3)

Means followed by distinct letters are significantly different (Tukey's test: SH, SH_1, SH_2 and $\%SH_R$. Dunn's test: ΔKHN. p<0.05). SH: surface hardness (baseline); SH_1: post-demineralization surface hardness; SH_2: surface hardness after pH cycling.

Positive control showed a lower remineralization rate of subsurface lesion (at 30 µm) when compared with acidic dentifrice with 412, 550 and 1,100 µgF/g. All experimental dentifrices with fluoride promoted remineralization of subsurface lesion.

Discussion

Many efforts have been made to reduce fluoride concentration in dentifrices without losing their anticariogenic efficacy, such as the use of trimetaphosphate (13). Acidic low fluoride dentifrices are able to keep the same fluoride concentration in dental plaque and to reduce fluoride ingestion in comparison to the conventional dentifrice (14). However, so far, there are no data regarding the ability of these dentifrices on improving enamel remineralization.

The possibility that acidic dentifrices might produce demineralization on enamel surface previously demineralized instead of enamel remineralization was discarded based on the increased values of SH_2 and $\%SH_R$ after pH cycling and treatment with low fluoride acidic dentifrices, even in placebo group. Moreover, the reduction of the dentifrices does not increase their abrasiveness (8). There was an increase on the remineralization process associated to the increase of fluoride concentration in dentifrices, showing a positive correlation between F in dentifrices and $\%SH_R$. The dose-response relationship found in the present study showed that the used pH cycling model is suitable to evaluate the remineralization capacity of acidic dentifrices with different F concentrations.

The current study used dentifrices with pH 4.5 and showed good results even in low F concentration. Dentifrices with 412 and 550 µgF/g showed similar or better results in comparison to that of positive control. These data suggest that pH reduction increased the reactivity between fluoride in dentifrices and enamel, in a mode of action similar to acidic fluoride gels: the acidified pH dissolves the superficial layers of enamel and the released calcium is precipitated as calcium fluoride (15). Calcium fluoride adsorbs to enamel surface acting as a fluoride reservoir, which, in turn, is released when the pH of the environment drops (16,17). The pH reduction of oral environment during the dentifrice treatment does not seem to affect fluoride bioavailability (18).

Comparing positive control and 1,100 µgF/g acidic dentifrice, there were no significantly differences in $\%SH_R$ values. On the other hand, ΔKHN was lower in group treated with 1,100 µgF/g acidic dentifrice. These results may be explained by the cross-sectional profiles, which showed a higher remineralization on the subsurface area of enamel (20-70 µm) of the 1,100 µg F/g dentifrice when compared with the positive control. The pH reduction increases CaF_2 deposition on enamel (5,19), promoting phosphorus incorporation in the enamel structure (20). During pH cycling, a higher CaF_2 formation increases the formation of fluoride reservoirs on enamel surface, which increases $\%SH_R$ and mineral gain by subsurface lesion. Moreover, CaF_2 formation depends on calcium and phosphate availability in environment (21). In the present study, phosphoric acid is added to dentifrices to reduce the decrease of pH. In the present study, phosphoric acid was used in the acidified toothpastes, which led to different phosphorus concentrations in the dentifrices. However, it is unlikely that phosphorus itself is responsible for the better anticariogenic action of the acidified toothpastes because only a mild correlation was found between $\%SH_R$ ($r=0.56$) or ΔKHN ($r=-0.35$) and P concentration ($p>0.05$). Moreover, Brighenti et al. (7) found that the positive control toothpaste contained 10-fold more phosphorus than the acidified toothpastes, but the 550 and 1,100 µg F/g experimental pastes showed similar results. In the present study, the 1,100 µgF/g acidic dentifrice presented 3 times more phosphorus concentration than 412 and 550 µgF/g acidic dentifrice; however they showed the same remineralization rate.

The increase in the amount of phosphorus concentration with the increase of fluoride concentration in dentifrices is directly related to the fact that the addition of NaF increases the product´s pH to around 8.0. Thus, a higher amount of phosphoric acid is needed to set the pH to 4.5 in dentifrices with 1,100 ppm F, as also observed by Brighenti et al. (6). However, the low fluoride dentifrices (412 and 550 ppm F) showed similar results when compared with the 1,100 acidulated dentifrice, which corroborates to the hypothesis that low pH - and not the amount of phosphorus - is responsible for the better performance of acidic toothpastes, as stated earlier (6).

Previous studies have shown that enamel microhardness values do not have a linear correlation with mineral content (22,23). Thus, in the present study, the authors did not convert the hardness values into mineral content. Instead, the integrated loss of subsurface hardness (ΔKHN) was calculated. Despite this limitation, hardness evaluation has the advantage of providing additional information, such as mechanical properties and structural integrity, which cannot be obtained by mineral content assessment (22).

Literature does not show advantages of using acidic dentifrices compared with neutral dentifrices. However, the results found in the present study demonstrated that low pH improves remineralization capacity of dentifrices with reduced fluoride concentration. The European Academy of Pediatric Dentistry recommends that dentifrices with low fluoride concentration should be used twice a day by children aged 2 to 6 years (24). The manufacture of low

fluoride acidic dentifrice can be stimulated by several studies in the literature that demonstrate favorable results when compared with a standard dentifrice (6,7,25). In a clinical trial, the low fluoride acidic dentifrice demonstrated similar effectiveness to that of a standard neutral dentifrice in high-caries-risk children living in a fluoridated area (25).

The results of this study are useful to support further clinical and/or *in situ* studies. They encourage the benefits of reducing fluoride content in dentifrices combined to pH reduction with no further prejudice of remineralization properties, especially to children at high risk for the development of dental fluorosis. Based on the outcomes, it may be concluded that acidic dentifrice (pH 4.5) with 550 µgF/g showed similar capacity to promote enamel remineralization as that of a standard dentifrice.

Resumo

O presente estudo objetivou avaliar a capacidade de dentifrícios fluoretados acidulados (pH 4,5) em promover a remineralização do esmalte utilizando um modelo de ciclagem de pH e compará-lo a um dentifrício padrão (1.100 µgF/g). Blocos de esmalte tiveram suas superfícies polidas e a dureza de superfície determinada (SH). Em seguida, foram submetidos à desmineralização subsuperficial e a dureza de superfície pós-desmineralização foi determinada. Os blocos foram divididos em seis grupos experimentais (n=10): placebo (controle negativo), 275, 412, 550, 1.100 µgF/g e um dentifrício padrão (controle positivo). Os blocos foram submetidos à ciclagem de pH durante seis dias e tratamentos com dentifrício diluído duas vezes por dia. Após a ciclagem de pH, a dureza de superfície e em secção transversal foram avaliadas para obtenção da porcentagem de recuperação de dureza de superfície ($\%SH_R$) e área integrada da perda de dureza de subsuperfície (ΔKHN). Os resultados mostraram que $\%SH_R$ foi semelhante entre os dentifrícios ácidos 412, 550, 1.100 µgF/g e controle positivo (teste de Tukey; p>0,05). Para ΔKHN, o dentifrício acidulado com 550 µgF/g mostrou uma performance melhor quando comparado ao controle positivo. Conclui-se que os dentifrícios acidulados 550 µgF/g apresentaram capacidade de remineralização semelhante ao controle positivo.

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