Anticaries Potential of Low Fluoride Dentifrices Found in The Brazilian Market

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Low-fluoride (F) dentifrices (<600 µg F/g) are widely available worldwide, but evidence to recommend the use of such dentifrices, with either regular or improved formulations, is still lacking. Therefore, the aim of this study was to evaluate the anticaries potential of low-F dentifrices found in the Brazilian market, using a validated and tested pH-cycling model. Enamel blocks were selected by surface hardness (SH) and randomized into four treatment groups (n=12): non-F dentifrice (negative control), low-F dentifrice (500 µg F/g), low-F acidulated dentifrice (550 µg F/g) and 1,100 µg F/g dentifrice (positive control). The blocks were subjected to pH-cycling regimen for 8 days and were treated 2x/day with dentifrice slurries prepared in water (1:3, w/v). The pH of the slurries was checked, and only the acidulated one had low pH. After the pH cycling, SH was again determined and the percentage of surface hardness loss was calculated as indicator of demineralization. Loosely- and firmly-bound F concentrations in enamel were also determined. The 1,100 µg F/g dentifrice was more effective than the low-F ones to reduce enamel demineralization and was the only one that differed from the non-F (p<0.05). All F dentifrices formed higher concentration of loosely-bound F on enamel than the non-F (p<0.05), but the 1,100 µg F/g was the only one that differed from the non-F in the ability to form firmly-bound F. The findings suggest that the low-F dentifrices available in the Brazilian market, irrespective of their formulation, do not have anticaries potential.

Key Words: fluorides, toothpaste, dental caries, demineralization

Introduction

The importance of fluoride (F) dentifrices to the decline of dental caries that occurred in the last decades is clearly established (1,2). On the other hand, their use has been pointed out as a risk factor for dental fluorosis (3). As an alternative to reduce the risk of fluorosis from dentifrices, low-F formulations (< 600 µg F/g) have been suggested. However, considering the best evidence available, they are not effective to satisfy the binomial anticaries effect by the use of F dentifrice with a lower risk of fluorosis (4). Moreover, systematic reviews focusing on caries prevention in permanent and deciduous teeth (5,6) showed that a dentifrice should have at least 1,000 µg F/g to provide significant anticaries effect.

Nevertheless, low-F dentifrices are available in many countries. Although in the United States they cannot be sold, because the American legislation (7) requires a minimum amount of soluble F of at least 650 ppm F for sodium fluoride (NaF) or 800 ppm F for monofluorophosphate (MFP) formulations, the European (8) and Mercosur (9) regulations only state the maximum F concentration that a dentifrice must contain (1,500 µg F/g). Similarly, the Brazilian regulatory agency (ANVISA) (10) only requires that a dentifrice has a maximum of 1,500 µg F/g, without mentioning the concentration of

soluble F or the effectiveness of the formulations based on the best available evidence (11). Low-F dentifrices with modified formulations have been developed to improve their anticaries effect with lower fluorosis risk (12-15). However, there is still no clinical data available that low-F dentifrices, irrespective of the formulation, are as effective as those with standard concentration (1,000-1,500 µg F/g) to reduce dentine caries without fluorosis risks. Therefore, the aim of this study was to assess the anticaries potential of low-F dentifrices available in the Brazilian market. The inhibition of enamel demineralization of two low-F dentifrices (500-550 µg F/g), with conventional or modified formulations, was compared with a standard dentifrice (1,100 µg F/g) using a validated pH-cycling model.

Material and Methods

Experimental Design

An *in vitro* pH-cycling model validated for the test of low-F dentifrices was used (16). The experimental units were bovine enamel blocks selected by surface hardness (SH) values and randomized into four treatment groups (n = 12): non-F dentifrice (Cocoricó®; Bitufo, Barueri, SP, Brazil) as a negative control, 500 µg F/g dentifrice (Oral-B Pro-Saúde Stages®, Procter & Gamble, Naucalpan, Mexico; importado por Procter & Gamble do Brasil SA, Queimados,

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RJ, Brazil), 550 µg F/g acidulated dentifrice (Escovinha®: Oralls, Dentalprev Ind. e Com. Ltda, Lorena, SP, Brazi) and 1,100 µg F/g dentifrice (Tandy®; Colgate-Palmolive Industrial Ltda, São Bernardo do Campo, SP, Brazil), as a positive control. All dentifrices were bought in the Brazilian market and were within the expiration time. They were NaF/silica-based and F concentration was checked in all dentifrices through analysis with ion selective electrode (17). The enamel blocks were subjected to daily pH cycles for 8 days; treatments with dentifrices slurries were made twice a day, before and after the demineralization cycle. The pH of the slurries and their fluoride concentration was checked immediately after preparation. After pH-cycling, the percentage of SH loss (%SHL) was calculated. Fluoride concentration in enamel (µg F/cm^2) as loosely- and firmly-bound F was determined. F concentration in the de-remineralization solutions (µg F/mL) was also analyzed.

Enamel Block Preparation

Bovine enamel blocks (4 x 4 x 2 mm) were flattened, polished and baseline SH was determined using a FM-ARS microhardness tester (Future Technology Corp., Kanagawa, Japan) with a Knoop diamond indenter under a 50-g load for 5 s. Three indentations, spaced 100 µm from each other, were made on the central area of each block and used to calculate SH, whose values were averaged. Forty-eight enamel blocks with hardness of 306.7 $\pm$ 7.9 kg/mm^2 were selected for this study.

Treatments and pH-Cycling Regimen

An adhesive tape was placed in the center of the enamel surface and the remaining surfaces of the block were coated with an acid-resistant varnish (Risqué®, Barueri, SP, Brazil). After removal of the tape, an area of enamel of 8.0 mm^2 (4 x 2 mm) was left to be exposed to the treatments.

The pH-cycling regimen (16) consisted of daily 4-h exposure to the demineralizing solution and approximately 20-h exposure to the remineralizing solution, at 37°C, for 8 days. Twice a day (before and after immersion in the demineralizing solution), the blocks were treated with a 1:3 (w/v) slurry of the dentifrices in purified water, for 5 min, under agitation (60 rpm), at room temperature, to simulate *in vivo* dentifrice exposure during toothbrushing (18). Before and after the treatments, the blocks were washed with purified water for 20 s and dried with soft paper. The demineralizing solution (pH 5.0) consisted of a 0.05 mol/L acetate buffer, containing 1.28 mmol/L Ca, 0.74 mmol/L P and 0.03 µg F/mL. The remineralizing solution (pH 7.0) was 0.1 mol/L TRIS buffer, containing 1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl and 0.05 µg F/mL. The proportion of de-remineralizing solutions per area of exposed enamel surface was 6.25 and 3.12 mL/mm^2, respectively. After the

4th day, the de-remineralizing solutions were replaced by fresh batches. After the 8th day of the pH-cycling regimen, the blocks were kept in the remineralizing solutions for 24 h until analysis.

Soluble F concentration in dentifrices slurries was measured in triplicate, using an ion-selective electrode (Orion 96-09) and an ion analyzer (Orion EA-940), previously calibrated with F standards containing 0.5 to 32.0 µg F/mL according to a previously described method (17). The results were expressed as µg F/mL. pH of the slurries was checked in four different samples of each dentifrice, immediately after the preparation of the slurries for use in the pH-cycling.

Enamel Demineralization Assessment

After the pH-cycling, the SH of the enamel blocks was measured again, as described above, and the %SHL was calculated [% SHL = 100 x (sound enamel hardness – hardness after pH-cycling) /sound enamel hardness]. SH is a validated technique to estimate mineral loss or gain by enamel because it reflects the demineralization degree of the enamel lesion (19).

Determination of Enamel Loosely- and Firmly-Bound Fluoride

After surface hardness analysis, the area of the blocks, which were covered by acid resistant varnish, was cut out and the remaining section was longitudinally sectioned through the center. The cut surfaces were isolated with wax leaving only a 4-mm^2 area (2 x 2 mm) of the enamel surface exposed for F enamel analysis.

Each enamel block was immersed in 0.15 mL of 1 M KOH for 24 h under agitation. After this period, the extract was buffered with an equal volume of TISAB II containing 1 M HCl. The concentration of loosely-bound F was determined in the extract using an ion-selective electrode (Orion 96-09; Orion Research, Inc., Boston, MA, USA) and an ion analyzer (Orion EA-940; Orion Research, Inc.) previously calibrated with F standards containing 0.1 to 8.0 µg F/mL. The results were expressed as µg F/cm^2 of enamel area.

After loosely-bound fluoride extraction, the blocks were immersed in 0.25 mL of 0.5 M HCl for 30 s under agitation. After this period, the extracts containing the dissolved enamel layer were buffered with an equal volume of TISAB II modified with 20 g of NaOH/L. The concentration of firmly-bound fluoride was determined as described above, against standards containing 0.125 to 4.0 µg F/mL. The results were expressed as µg F/cm^2 of enamel area.

Determination of Fluoride Concentration in the De- and Remineralizing Solutions

Fluoride concentration in the de-remineralizing

solutions was checked immediately after preparation. After the pH-cycling, all the solutions in which the blocks were individually immersed were again measured for fluoride concentration. Solutions were buffered with TISAB III and F concentration determined using an ion-selective electrode (Orion 96-09) and an ion analyzer (Orion EA-940) previously calibrated with fluoride standards containing 0.025 to 0.4 µg F/mL.

Statistical Analysis

The equality of variances and a normal distribution of error were checked for all response variables. The %SHL, loosely- and firmly-bound F data were transformed to the $\log_{10}$. ANOVA was used for all analysis, followed by Tukey test. The SAS System 9.0 software (SAS Institute Inc., Cary, NC, USA) was used and the significance level was set at 5%.

Results

Fluoride concentration and pH of the dentifrices slurries are presented in Table 1.

After the pH-cycling regimen (Table 2), all groups presented a decrease of surface hardness, which was significantly lower for the positive control (p<0.05). Similarly, only blocks treated with the positive control presented significantly higher firmly-bound F concentration after the cycling than the negative control group (p<0.05). The low-F dentifrices did not differ significantly from the negative control regarding the %SHL and firmly-bound F concentration (p>0.05). The concentration of loosely-bound F was higher in all F groups when compared with the negative control (p<0.05), being the highest for the acidulated dentifrice.

All F groups were able to significantly increase F concentration in the de- and remineralizing solutions when compared with the negative control (p<0.05) (Table 3). This effect was higher for the group treated with the acidulated dentifrice.

Discussion

F present in dentifrices acts as a preventive-therapeutic agent (20), but the currently available evidence suggests that they must have at least 1,000 µg soluble F/g to be able to significantly control caries in permanent (5)

Table 1. Soluble fluoride (F) (mean ± SD, n=3) and pH (mean ± SD, n=4) of the prepared dentifrice slurries

Dentifrice	Soluble fluoride (µg F/mL)	pH
Non-F	1.6 ± 0.2	7.02 ± 0.02
Low-F	144.8 ± 12.8	7.57 ± 0.07
Low-F acidulated	143.3 ± 3.4	4.37 ± 0.08
Standard	314.7 ± 7.2	7.27 ± 0.09

Non-F dentifrice: (negative control - Cocoricó®, Bitufo); Low-F dentifrice 500 µg F/g (Oral-B Pro-Saúde Stages®, Procter & Gamble); Low-F acidulated dentifrice: 550 µg F/g (Escovinha®, Oralls, Dentalprev); Standard F dentifrice: 1100 µg F/g (positive control - Tandy®, Colgate).

Table 2. Enamel surface hardness loss (%SHL) and fluoride (F) concentration in enamel after pH-cycling (mean ± SD, n=12)

Dentifrice	%SHL	F concentration (µg F/cm²)	
		Loosely-bound F	Firmly-bound F
Non-F	35.9 ± 8.9 a	0.3 ± 0.1 a	2.6 ± 0.8 a
Low-F	30.8 ± 11.1 a	0.6 ± 0.2 b	2.8 ± 1.2 a
Low-F acidulated	30.2 ± 6.6 a	1.1 ± 0.2 c	2.9 ± 1.1 ab
Standard	15.9 ± 5.2 b	0.6 ± 0.3 b	4.8 ± 2.4 b

Non-F dentifrice: (negative control - Cocoricó®, Bitufo); Low-F dentifrice 500 µg F/g (Oral-B Pro-Saúde Stages®, Procter & Gamble); Low-F acidulated dentifrice: 550 µg F/g (Escovinha®, Oralls, Dentalprev); Standard F dentifrice: 1100 µg F/g (positive control - Tandy®, Colgate). Means followed by distinct letters differ significantly (p<0.05).

Efficacy of low fluoride dentifrice

Table 3. Fluoride (F) concentration in de-remineralizing solutions after the pH cycling (mean ± SD, n=12)

Dentifrice	Demineralizing solution (µg F/mL)		Remineralizing solution (µg F/mL)	
	1st cycling period	2nd cycling period	1st cycling period	2nd cycling period
Non-F	0.035 ± 0.001 a	0.035 ± 0.001 a	0.040 ± 0.001 a	0.038 ± 0.001 a
Low-F	0.036 ± 0.001 b	0.037 ± 0.001 b	0.049 ± 0.001 b	0.050 ± 0.002 b
Low-F acidulated	0.042 ± 0.002 c	0.048 ± 0.002 c	0.061 ± 0.003 c	0.076 ± 0.008 c
Standard F	0.036 ± 0.001 b	0.038 ± 0.001 b	0.049 ± 0.002 b	0.050 ± 0.002 b

Non-F dentifrice: (negative control - Cocoricó®, Bitufo); Low-F dentifrice 500 µg F/g (Oral-B Pro-Saúde Stages®, Procter & Gamble); Low-F acidulated dentifrice: 550 µg F/g (Escovinha®, Oralls, Dentalprev); Standard F dentifrice: 1100 µg F/g (positive control - Tandy®, Colgate). Means followed by distinct letters differ significantly (p<0.05).

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and deciduous teeth (6). However, the current Brazilian legislation (10) on fluoride dentifrices only determines the maximum F concentration that a dentifrice must contain, without specifying that it should be soluble or requiring a minimum concentration. This raises two concerns: 1. Depending on the dentifrice formulation, not all fluoride is soluble (11,17,21), specially as dentifrices ages (22), suggesting that the concentration of soluble F should be considered in the legislation (11); 2. If low-F dentifrices are used, the concentration of F used does not reach the recommended 1,000 μg F/g. In addition, currently there is no evidence that using a low-F dentifrice reduces the risk of dental fluorosis (4). Therefore, considering the availability of low-F dentifrices in the Brazilian market, we aimed to test their anticaries potential.

The results showed that only the standard F dentifrice (1,100 μg F/g) was able to significantly reduce mineral loss during the pH-cycling. This result is confirmed by the firmly-bound F concentration found after the pH-cycling, indicating the F that was incorporated in enamel as a result of the caries process (20). This finding suggests that the highest F concentration present in the standard dentifrice provided higher incorporation of fluorapatite in the enamel during the de-remineralization cycles. In this process, part of the dissolved minerals was replaced in the enamel in the form of a more stable mineral, slowing the mineral loss.

On the other hand, the loosely-bound F concentration found on enamel after the pH-cycling showed that the reduction in pH in the low-F dentifrice was able to enhance the formation of this material (calcium-fluoride like) on enamel. This result was expected given that the formation of calcium fluoride reservoirs on the enamel increases with lowering the pH of the fluoridated agent (23). In fact, previous study (24) confirmed that lowering the pH of a low-F dentifrice may enhance its reactivity with enamel to values similar to those of a standard F dentifrice.

Taking into account the solubility of calcium fluoride deposits formed on the dental structure (25), the greater reactivity of the acidulated dentifrice resulted in higher F release for de-remineralizing solutions, according to the results obtained by Brighenti et al. (12). However, the higher reactivity of acidulated dentifrice and the subsequent higher fluoride concentration released to the solutions did not reduce the mineral loss. F released to the solutions was not sufficient to facilitate the incorporation of fluorapatite in enamel, limiting the anticaries potential of the acidulated low F dentifrice. These results differed from those obtained by Brighenti et al. (12) and Alves et al., (13), who showed that acidic formulations (550 μg F/g) had similar anticaries effect than neutral formulations (1,100 μg F/g) in reducing enamel demineralization. The discrepancy of results can be explained by the ratio volume of de-remineralizing

solutions per area of enamel exposed. We used a 3 times higher ratio, as recommended (26), avoiding that F released from the loosely-bound reservoirs formed by the low-F acidulated dentifrices had accumulated in the pH-cycling solutions. In fact, the difference of fluoride concentration in de-remineralizing solutions for the different treatments is very small (Table 2). Therefore, the ratio volumes of de-remineralizing solutions per area of enamel avoid artifacts and allow a better simulation of the mouth open system. Thus, in order to simulate the continuous dilution promoted by saliva in a closed in vitro design, the volume of the solutions must be high enough to avoid accumulation of the anticaries agents being tested due to the closed in vitro model (26). Indeed, our findings are supported by a previous study showing that loosely-bound F reservoirs are not able to explain how fluoride from dentifrice works to control caries (27).

The results of the present study emphasizes the importance of a minimum F concentration in dentifrices for a significant anticaries effect, since the higher reactivity of the low pH formulation was not able to reduce mineral loss when compared with the negative control. Although the acidulated dentifrice has released the highest concentration of fluoride to the de-remineralizing solutions, this increase was minimal showing no significant effect in preventing mineral loss compared to the effect provided by the fluoride concentration present in the positive control dentifrice. Additionally, it is important to consider that the dentifrice slurries were prepared with water, which maintains the low pH of the acidulated dentifrice slurries and consequently promotes higher reactivity with dental structure. However, when the acidulated formulation slurry was prepared using artificial saliva, the pH was higher than 6 (data not shown), suggesting that in vivo it would be even less effective to form loosely-bound F reservoirs on enamel.

In conclusion, the present in vitro study confirmed that a dentifrice must have at least 1,000 μg F/g to control caries, since none of the low-F dentifrices found in the Brazilian market were able to significantly reduce mineral loss.

Resumo

Dentifrícios de baixa concentração de fluoreto (F) (< 600 μg F/g) estão amplamente disponíveis no mundo, mas ainda não há evidência para recomendar seu uso, quer seja em formulações regulares ou melhoradas. Assim, o objetivo deste estudo foi avaliar o potencial anticárie de dentifrícios de baixa concentração de fluoreto encontrados no mercado brasileiro, utilizando um modelo de ciclagens de pH validado e testado. Blocos de esmalte bovinos foram selecionados pela dureza de superfície e randomizados em quatro grupos (n=12): dentifrício sem fluoreto (controle negativo), dentifrício de baixa concentração de fluoreto (500 μg F/g), dentifrício acidulado de baixa concentração de fluoreto (550 μg F/g) e dentifrício de 1100 μg F/g (controle positivo). Os blocos foram submetidos ao regime de ciclagem de pH por 8 dias e tratados 2 x/dia com suspensões aquosas dos dentifrícios (1:3 p/v). O pH das suspensões foi checado, e apenas o acidulado tinha baixo pH. Após a ciclagem de

pH, a dureza de superfície foi novamente determinada e a porcentagem de perda de dureza foi calculada como indicador de desmineralização. As concentrações de fluoreto fracamente e firmemente ligado ao esmalte também foram determinadas. O dentifrício de 1.100 µg F/g foi mais efetivo do que os de baixa concentração na redução da desmineralização do esmalte e foi o único que diferiu significativamente do não fluoretado (p<0,05). Todos os dentifrícios fluoretados foram capazes de formar maiores concentrações de fluoreto fracamente ligado ao esmalte do que o não fluoretado (p<0,05), mas o de 1.100 µg F/g foi o único que diferiu do não fluoretado na capacidade de formar fluoreto firmemente ligado ao esmalte. Os resultados sugerem que dentifrícios de baixa concentração de fluoreto disponíveis no mercado brasileiro, independentemente da formulação, não têm potencial anticárie.

Acknowledgements

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