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Mechanisms of action of fluoridated acidic liquid dentifrices against dental caries

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ABSTRACT

Objective: This study attempted to clarify the mechanisms of action of fluoridated acidic liquid dentifrices against dental caries.

Design: In the in vitro leg, enamel specimens were submitted to a pH-cycling model, treated with distinct dentifrices (0, 550 μgF/g pH 4.5 and pH 7.0, 1100 or 5000 μgF/g pH 7.0) and analyzed using hardness. Alkali-soluble fluoride (F) deposition was quantified on pre-demineralized specimens treated with the dentifrices. In the clinical leg, 2-to-4-year-old children who had been using liquid dentifrices for 6 months (550 μgF/g pH 4.5 or pH 7.0 or 1100 μgF/g pH 7.0) had their plaque samples collected 5 and 60 min after the last brushing. Fluoride uptake in whole plaque was evaluated.

Results: The reduction of the pH had a partial preventive effect on subsurface hardness loss only. [F] had a significant influence on the deposition of fluoride, surface and subsurface hardness loss. In vivo, the reduction of the pH was able to significantly increase plaque F uptake, leading to similar levels as those found for the neutral dentifrice containing twice [F].

Conclusion: The results obtained from in vitro studies whose design does not include the presence of dental plaque should be interpreted with caution.

1. Introduction

The reduction of fluoride (F) intake by children is an important strategy to minimize the risk of dental fluorosis occurrence.[1–4] In early childhood, the use of conventional 1100 μgF/g dentifrice significantly contributes to the daily F ingestion dose.[2] Therefore, a possible alternative would be the use of low-F (around 500 μgF/g) dentifrices. However, only few studies assessed the anticaries effect of low-F dentifrices in

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the primary dentition and there is still uncertainty about the effectiveness of low-F dentifrices to prevent caries when compared to conventional ones (around 1000 μgF/g).[4,5] At this age range, excessive F intake could lead to dental fluorosis in permanent maxillary central incisors.[3] Thus, strategies have been proposed to increase the anticaries efficacy of low-F dentifrices such as the addition of sodium trimetaphosphate[6–9] and the pH reduction.[10–13] In vitro studies have shown that low-F (500 μgF/g) acidic (pH 4.5–5.0) dentifrices are able to reduce the enamel mineral loss.[11,13] The decrease of the pH also tends to enhance salivary F concentrations shortly after brushing, without changing F bioavailability.[14]

In order to reduce the amount of dentifrice loaded onto the toothbrush, recently a low-fluoride (500 μgF/g) acidic (pH 4.5) liquid dentifrice (LD) was developed and tested in two clinical trials. The first was conducted with 4-year-old children who used the dentifrices for 20 months. The dentifrices were applied using the "drop" technique,[15] which reduces the amount of F ingested, as reflected by the reduced nails F concentrations. The 550 μgF/g acidic LD led to similar caries progression rates (dmfs) as the 1100 μg/g F liquid and conventional dentifrice.[16] The pH and fluid consistency of the dentifrice led to increased F uptake in whole plaque, despite the difference was not significant among the LDs (550 μgF/g acidic, 1100 μgF/g acidic and 1100 μgF/g neutral dentifrices).[17] In the second clinical trial, the effect of pH and [F] of LDs on caries progression of 2–4-year-old children was assessed after 12 months. Children living in a fluoridated area, with or without active caries lesions, were randomly allocated into three groups according to the LD used: G1 – 550 μgF/g – pH 4.5; G2 – 1100 μgF/g – pH 7.0 and G3 – 550 μgF/g – pH7.0. Caries net increment followed a decreasing pattern according to the dentifrice used (G1 < G2 < G3), regardless caries activity. Significant differences were detected only for caries progression and net increment (G1 < G3) for the caries-active group when evaluated through visual inspection. QLF analysis detected no significant difference between G1 and G2, but they performed significantly better than G3. A significantly lower toenail fluoride concentration was observed when children used the low-F dentifrices.[18]

Information is lacking regarding the mechanisms by which the reduction of the pH could increase the anticaries efficacy of dentifrice. One possible hypothesis (HYPOTHESIS 1) could be the increased formation of CaF_2-like deposits on enamel.[19] These deposits have been recognized as the most prominent labile sources of F on the dental surface, releasing F upon cariogenic challenges.[20–22] However, it is not known if significantly higher amounts of CaF_2-like deposits would be formed by the use of low-F acidic LD.

On the other hand, another plausible hypothesis would be the potential of acidic dentifrices to enhance plaque F uptake (HYPOTHESIS 2), as was observed in previous studies.[12,17] The clinical efficacy of topical fluoridated products is directly related to the intraoral F levels and plaque is an important fluoride reservoir. Plaque F reservoirs can be divided into two broad types, both of them involving calcium (Ca): the mineral calcium fluoride (CaF_2) and biologically/bacterially bound calcium fluoride deposits (–Ca–F), in which F is held by Ca ions bound on the surface of these entities.[23] The F bound on bacteria seems to be the most important F reservoir in plaque.[22] Calcium binding is predominantly phosphate group-based, especially in lipoteichoic and teichoic acid presented in the bacteria surface.[24] Release of F, bound by calcium bridging, into plaque fluid, as a result of salivary F clearance or of a fall in pH, will always be accompanied by a calcium release, which will potentiate the cariostatic effect of F.[23]

Based on the above considerations, this study was developed in attempt to gain insight into the mechanisms by which low-fluoride acidic LD could protect against caries. For this purpose, *in vitro* experiments to test HYPOTHESIS 1 were combined with a clinical leg that took advantage of an ongoing clinical trial to test HYPOTHESIS 2.

2. Materials and methods

2.1. *In vitro leg*

2.1.1. *Preparation of the enamel specimens*

Enamel specimens (4 mm × 4 mm × 3 mm) were prepared from bovine incisor teeth, polished and cleaned as described earlier.[25]

2.1.2. *Treatment and pH-cycling*

Sixty sound enamel specimens with an average baseline hardness of 340.10 ± 19.4 Kg/mm^2 were randomly distributed into five groups: experimental LD with 550 μgF/g (pH 4.5); 550 μgF/g (pH 7.0); 1100 μgF/g (pH 7.0); 5000 μgF/g (pH 7.0); and placebo (non-F control, pH 7.0). The experimental dentifrices were NaF-based and had silica as abrasive. Their composition is described in a previous study[17] and the abrasiveness was reported in the study of Moron et al.[26] The fluoride concentration was assessed using a specific electrode (Orion 9409) and potentiometer (Orion EA940). The pH values were directly checked using indicator strips (Whatman, GE, USA).

The sound enamel specimens were subjected to a pH-cycling model, during 7 days.[27] During 5 days, the specimens were immersed in demineralizing solution [2.0 mmol L^{-1} $Ca(NO_3)_2{\cdot}4H_2O$, 2.0 mmol L^{-1} $NaH_2PO_4{\cdot}2H_2O$, 0.075 mol L^{-1} acetate buffer, 0.04 ppm F, pH 4.7] for 6 h and in remineralizing solution [1.5 mmol L^{-1} $Ca(NO_3)_2{\cdot}4H_2O$, 0.9 mmol L^{-1} $NaH_2PO_4{\cdot}2H_2O$, 150 mmol L^{-1} KCl, 0.02 mol L^{-1} Tris buffer, 0.05 ppm F, pH 7.0] for 18 h. They were exposed to the dentifrices two times daily, immediately before (time point: 0) and after demineralization (time point: 6 h). The dentifrices were directly placed onto the toothbrushes (~0.15 g),[15] and then the specimens were brushed using an electrical toothbrush (Colgate Motions Multi-action, Brazil) for 15 s (166 oscillations/s, 1.5 N, 25 °C). After treatment, the specimens were rinsed in water for 5 s. In the last 2 days, the specimens were maintained only in remineralizing solution.

2.1.3. *Hardness determination*

Surface hardness was measured at baseline and at the end of the experiment. Five indentations were performed, 100 μm apart from each other, using 25 g for 10 s (Knoop, Microhardness tester Shimadzu HMV-2, kg/mm^2) to calculate the percentage of surface hardness change. To perform cross-sectional hardness (CSH) tests, the specimens were longitudinally sectioned through the centre, embedded and polished. Three rows of seven indentations each were made, one in the