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In vitro effect of low-fluoride toothpastes containing sodium trimetaphosphate on enamel erosion

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ABSTRACT

Objective: To evaluate the effect of a low-fluoride dentifrice (LFD) containing sodium trimetaphosphate (TMP) on enamel erosion *in vitro*.

Design: Bovine enamel blocks ($n = 144$) were selected by surface hardness (SH) and subjected to erosive challenges, in two sets of experiments for 2 and 5 days. Blocks were randomly assigned to groups treated with slurries (5 mL/block, for 15 s) of following dentifrices: Placebo (no fluoride or TMP); LFD (250 ppm F); LFD plus 0.25, 0.5, or 1.0% TMP; and a commercial positive control (1,425 ppm F). The erosive challenge was produced by immersion in a soft drink (pH 2.8) for 5 min, four times/day, interspersed by immersion in artificial saliva for 1 h. SH and surface wear were analyzed as response variables. Data were submitted to 2-way ANOVA, followed by Student–Newman–Keuls test ($p < 0.05$).

Results: All groups treated with LFDs containing TMP had significantly lower enamel wear when compared with the other groups tested ($p < 0.001$). Also, the LFDs containing TPM at lower concentrations promoted SH similar to the commercial positive control, both being significantly higher than the LFD without TMP and Placebo ($p < 0.001$).

Conclusion: The supplementation of LFDs with TMP is able to significantly increase the anti-erosive potential of these formulations *in vitro*.

1. Introduction

Dental erosion has become an increasingly common dental problem, with incidence reported to vary widely among individuals of all age groups.[1] Tooth erosion is a multifactorial condition and over time the interaction of factors related to the patient and/or to the dietary habits may lead to either progression of the lesion or to the protection of the surface.[2] The terms erosion and erosive tooth wear have been suggested to refer to the chemical and chemical–mechanical processes, respectively.[3]

As for dental caries and periodontal disease, changes in oral health and dietary habits, as well as the use of products for oral care have been recommended to prevent or reduce the progression of dental erosion.[4] Fluoride has been used as a supplementary preventive measure to decrease the progression of erosion, and it has been demonstrated to have superior effect when applied at high concentrations.[5–8] Topical fluoride is, however, most frequently applied

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as toothpaste, whose effects on enamel erosion are contradictory.[9–13]

The addition of non-fluoride active compounds aiming to increase the effectiveness of topically applied formulations against enamel erosion has been studied in toothpastes. Xylitol and fluoride were demonstrated to have a synergistic effect on enamel protection against erosion, possibly due to the ability of xylitol in facilitating calcium movement and availability in demineralized enamel,[14,15] despite different results were found using a different research protocol.[16] Some commercial toothpastes have both fluoride and desensitizing agents (e.g., $SrCl_2$, potassium citrate, and KNO_3) and are claimed to prevent enamel erosion, although the association of NaF and KNO_3 did not produce favourable results *in vitro*.[17] Another possibility is the association between fluoride and inorganic phosphates,[18,19] among which sodium trimetaphosphate (TMP) has been shown to be effective in dentifrices, gels and mouth rinses against dental caries and erosion.[8,13,20,21]

Specifically regarding toothpastes, Moretto et al.[13] demonstrated that a dentifrice containing 500 ppm F supplemented with 3% TMP promoted a similar protective effect against enamel erosion in comparison with a 5,000 ppm F toothpaste, resulting in significantly lower wear compared with a 1,000 ppm F dentifrice. Moreover, the anti-caries effectiveness of a 250 ppm F toothpaste associated with 0.25% TMP has been shown to be similar to that obtained by the use of a conventional dentifrice (1100 ppm F) without TMP.[22] This association, however, has not yet been assessed for enamel erosion. Considering that the mechanism of action of TMP has not still been determined, it would be interesting to evaluate the effects of the association of a low-fluoride dentifrice (250 ppm F) supplemented with TMP also against enamel erosion.

Based on the studies assessing the protective effects of fluoridated vehicles supplemented with TMP against enamel erosion, it was hypothesized that enamel treated with a 250 ppm F dentifrice would have significantly lower wear when compared to its counterpart without TMP. The effects of the association between F and TMP were also assessed by surface hardness, in order to provide additional data that could contribute to a better understanding of the mechanisms of action of TMP on enamel.

2. Materials and methods

2.1. Experimental design

One hundred forty-four bovine enamel blocks were randomly assigned to treatment with the following toothpastes: Placebo (no fluoride or TMP); 250 ppm F; and 250 ppm F plus 0.25, 0.5, or 1.0% TMP; and a commercial toothpaste (1,425 ppm F). Blocks were further divided into 2 subgroups according to duration of erosive challenge (2 or 5 days). A sample size of 12 enamel blocks was calculated (SigmaPlot software version 12.0) considering an α-error level of 5% and a β-error level of 20%.[8,21] The erosive challenge was produced in all blocks by immersion in a soft drink (Sprite Zero™, pH 2.8) for 5 min, 4 times per day. Blocks were then analyzed by profilometry and by surface hardness.

2.2. Enamel sample preparation

Enamel blocks (4 × 4 mm) were obtained from bovine incisors and kept immersed in a 2% formalin solution (pH 7.0) for 30 days prior to experimental procedures. The enamel surface of the blocks was ground flat with water-cooled silicon carbide paper discs (400, 600, and 1200 grades; Buehler, Lake Bluff, Ill, USA) removing approximately 200 μm of the enamel surface.[8,13] After polishing, the baseline hardness of the enamel surface was determined and blocks with 330.0 to 370.0 Kgf/mm^2 were selected.[13] To maintain a reference surface for lesion-depth determination by profilometry, 2 layers of nail varnish were applied to half of the surface of each block.[8,21]

2.3. Toothpaste formulation and fluoride and pH analysis

The experimental toothpastes were produced in the Laboratory of Pediatric Dentistry, Araçatuba Dental School, São Paulo State University (Brazil) and contained the same basic ingredients: carboxymethyl cellulose, sodium methyl-*p*-hydroxybenzoate, sodium saccharin, peppermint oil, glycerol, hydrated silica, sodium lauryl sulphate, and water. To compare and validate the results, the following toothpastes were assembled: Placebo (without F or TMP); low-fluoride dentifrice (LFD, 250 ppm F-NaF; Merck, Darmstadt, Germany); and LFDs with TMP (TMP; Sigma–Aldrich Co., St. Louis, MO, USA) added at concentrations of 0.25, 0.5, or 1.0%. A commercial toothpaste was also included as a positive control (Sensodyne Pronamel, 1,425 ppm F as NaF, 5% KNO_3, pH 7.0; GlaxoSmithKline Brazil Ltd., Rio de Janeiro, Brazil).

Total fluoride (TF) and ionic fluoride (IF) were determined using a specific electrode for F (Orion 9609-BN; Orion Research Inc., Beverly, MA, USA) according to the methods described by Delbem et al.[23]. Approximately 0.1 g of each toothpaste was suspended in 10.0 mL deionized water in a polypropylene tube (15 mL). For TF measurement, 0.25 mL of 2 mol/L HCl was added to 0.25 mL of the dentifrice suspension and agitated for 1 h, at 45 °C. Following, 0.5 mL 1.0 mol/L NaOH was added to the mixture. For IF, the dentifrice suspension was centrifuged at 906 × *g* for 20 min and 0.25 mL of the supernatant was treated with 0.25 mL 2 mol/L HCl and 0.5 mL 1.0 mol/L NaOH. TISAB II was added to the samples and to the standards and results were expressed in μg F/g.

The pH of the toothpaste slurries was analyzed in triplicate with a pH electrode (FH 2A09E-Analyser; São Paulo, SP, Brazil) and ion analyzer (Orion 720A^{plus}). The pH electrode was calibrated using pH standards at 7.0 and 4.0. Fluoride concentrations and pH of the toothpastes are shown in Table 1.

Table 1 – Mean values (SD, *n* = 3) of total (T) and ionic (I) fluoride (F) and pH in the toothpastes.

Groups	TF (μg F/g)	IF (μg F/g)	pH
Negative control	11.1 (1.4)	11.5 (0.8)	7.7 (0.02)
250 ppm F	268.7 (3.1)	272.3 (1.1)	7.1 (0.02)
250 TMP 0.25%	282.0 (2.8)	273.4 (6.8)	7.5 (0.01)
250 TMP 0.5%	270.6 (3.5)	277.3 (2.1)	7.3 (0.01)
250 TMP 1%	271.2 (0.9)	270.3 (6.7)	7.0 (0.02)
Positive control	1,578.2 (14.0)	1,432.2 (23.0)	7.3 (0.04)