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Short communication

Remineralizing effect of a fluoridated gel containing sodium hexametaphosphate: An *in vitro* study

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ABSTRACT

Objectives: To evaluate *in vitro* the effect of neutral pH topical gels with reduced fluoride concentration (F), supplemented or not with sodium hexametaphosphate (HMP) on the remineralization of dental enamel, using a pH-cycling model. Materials and methods Bovine enamel blocks with caries-like lesions were randomly treated with five gels (n = 24/group): without F/HMP (Placebo); 4500 ppm F (4500F), 4500F plus 9% HMP (4500F + HMP); 9000 ppm F (9000F); and 12,300 ppm F (Acid gel). After pH-cycling, the percentage of surface hardness recovery (%SHR), integrated loss of subsurface hardness (ΔKHN), and concentrations of loosely- (CaF_2) and firmly-bound (FA) fluoride formed and retained in/on enamel were determined. The results were analyzed by ANOVA followed by the Student-Newman-Keuls test ($p < 0.001$).
Results: The 4500F + HMP gel promoted the highest %SHR among all groups; the lowest ΔKHN was achieved by 4500F + HMP and Acid gel, without significant differences between these. The Acid gel group presented the highest CaF_2 and FA formed and retained on/in enamel ($p < 0.001$).
Conclusion: Based on the present results, the addition of 9% sodium hexametaphosphate to a gel with reduced fluoride concentration (4500F) was able to significantly enhance the remineralization of artificial carious lesions *in vitro* when compared to 4500F, reaching protective levels similar to an acidic formulation with ~3-fold higher fluoride concentration.

1. Introduction

Fluoride gels have been widely used as a caries-preventive measure in several countries, both for professional application or self-applied. Even though its clinical efficacy has been demonstrated for both primary and permanent dentitions, these products are not typically recommended for children under 6 years of age due to concerns related to acute toxicity resulting from product ingestion during application (Marinho, Worthington, Walsh, & Chong, 2015). To minimize the possibility of side-effects without compromising the therapeutic effect of gels, strategies to enhance the preventive and therapeutic effects of fluoride by the association with inorganic phosphates have been intensively studied over the last decade.

The addition of sodium hexametaphosphate (HMP) to toothpastes and gels with reduced fluoride concentration was shown to promote a synergistic protective effect against enamel demineralization *in vitro* (Danelon et al., 2012; da Camara, Miyasaki, Danelon, Sassaki, & Delbem, 2014). Sodium hexametaphosphate interferes with the enamel de-remineralization processes due to its ability to bind to the enamel surface and reduce its solubility. This phosphate also has antimicrobial activity, due to its ability to increase the permeability of the bacterial outer membrane (Vaara & Jaakkola, 1989), as well as inhibitory activity against biofilm formation (Shibata & Morioka, 2001). Considering that gels are also used as therapeutic vehicles for the reversal of non-cavitated caries lesions, the study of the remineralizing effect of sodium hexametaphosphate containing gels with reduced fluoride concentration could provide additional information on the real benefits of such formulations. Thus, this study assessed the remineralizing effect of a low-fluoride gel containing HMP *in vitro*, using a pH-cycling model. The null hypothesis was that the test gel would present a similar remineralizing effect when compared with its counterpart without HMP.

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2. Material and methods

2.1. Experimental design

Enamel blocks (4 mm × 4 mm, n = 120) were obtained from bovine incisors kept in formaldehyde (2%, pH 7.0) for 30 days prior to the experimental procedures (Danelon, Takeshita, Sassaki, & Delbem, 2013). The enamel surface of the blocks was serially polished and selected by surface hardness (SH, 369.0 to 377.0 KHN). The blocks were then demineralized (induction of subsurface lesions), analyzed by surface hardness after demineralization (SH1), and randomly assigned into five treatments (n = 24/group): without F/HMP (Placebo); 4500 ppm F (4500F); 4500F plus 9% HMP (4500F + HMP); 9000 ppm F (9000F); and 12,300 ppm F (Acid gel). The gels were applied only once (1 min). Half of the blocks (n = 12/group) were used for determination of loosely- (CaF_2) and firmly-bound (FA) fluoride formed on/in enamel. The other blocks (n = 12/group) were subjected to six pH cycles. The percentage of hardness surface recovery (%SHR) and cross-sectional hardness (ΔKHN) were assessed, and the concentrations of CaF_2 and FA retained on/in enamel were determined.

2.2. Gel formulation and determination of fluoride and pH in products

The experimental gels were prepared in the laboratory of Pediatric Dentistry, School of Dentistry, Araçatuba (UNESP, Brazil) using the following ingredients: 8.0 g carboxymethyl cellulose (Synth, Diadema, São Paulo, Brazil), 0.1 g sodium saccharin (Vetec, Duque de Caxias, Rio de Janeiro, Brazil), 28.0 g glycerol (Sigma-Aldrich Co., St. Louis, MO, USA), 0.5 g peppermint oil (Synth, Diadema, São Paulo, Brazil), and adjusted with deionized water to 100 g. Fluoride (NaF, Merck, Darmstadt, Germany) was added to the gel at concentrations of 4500 (1.0 g of NaF), or 9000 ppm F (2.0 g of NaF), besides a fluoride-free gel (Placebo, negative control). An HMP-containing gel was also produced by the addition of HMP (Sigma-Aldrich Co., St. Louis, MO, USA) at a concentration of 9% to the 4500 ppm F gel. A commercial acidic gel was used as positive control (12,300 ppm F, Acid gel, pH = 4.5; DFL Indústria e Comércio S.A., Rio de Janeiro, RJ, Brazil). The ionic fluoride (IF) concentration in the gels and the pH were determined as previously described (Danelon et al., 2013; Danelon, Takeshita, Peixoto, Sassaki, & Delbem, 2014). Mean (SD, n = 2) IF concentrations were 23.7 (4.1); 4,519.2 (41.0); 4,514.6 (22.7); 9,487.5 (18.2); and 12,889.0 (15.4), respectively for Placebo, 4500F, 4500F + HMP, 9000F, and Acid gel. The mean (SD) pH of the neutral gels was 6.4 (0.2), ranging from 6.0 to 6.7. The pH of the Acid gel was 4.0 (0.6).

2.3. Induction of subsurface lesions

All surfaces of each block, except for the enamel surface, were coated with acid-resistant varnish (Risqué®-Brazil), and subsurface enamel demineralization was produced by immersing each enamel disc in 32 mL of a solution with 1.3 mmol/L Calcium, 0.78 mmol/L Phosphorus in 0.05 mol/L acetate buffer, pH 5.0; 0.03 ppm F; for 16 h at 37 °C (Queiroz, Hara, Leme, & Cury, 2008). Thereafter, surface hardness after demineralization (SH1) was determined and the mean and standard deviation (SD) for all enamel blocks was 58.9 (14.7) KHN, the lowest mean (SD) value of the groups was 57.1 (22.6) and the highest was 65.5 (11.2), without significant differences between groups (p = 0.631).

2.4. Treatment with the gels and pH-cycling

The gels were applied for 1 min to each block (3 g/block) only once (Danelon et al., 2013), removed with gauze and washed with deionized water for thirty seconds. After that, twelve blocks from each group were immersed (8 a.m.) in the remineralizing solution (1.5 mmol/L Calcium, 0.9 mmol/L Phosphorus, 0.15 mol/L Potassium Chloride in 0.02 mol/L cacodylate buffer, 0.04 ppm F, pH 7.0; 4 mL/block), for 4 h. Following (12 p.m.), the blocks were washed with deionized water, gently dried and immersed in the demineralizing solution (2.0 mmol/L Calcium and Phosphorus in 0.075 mol/L acetate buffer, 0.03 ppm F, pH 4.7; 12 mL/block) for 2 h (12 p.m. to 2 p.m.). At 2 p.m., the blocks were washed and immersed in the same remineralizing solution previously used. At 4 p.m., the blocks were washed and immersed in a fresh remineralizing solution until 8 a.m. on the following day. The blocks were subjected to pH-cycling in individual vials for 6 days at 37 °C (Vieira et al., 2005). Fresh de- and remineralizing solutions were used every day.

2.5. Analysis of enamel hardness

Surface hardness (SH) was determined with Micromet 5114 hardness tester (Buehler, Lake Bluff, USA) and Buehler Omni Met software (Buehler, Lake Bluff, USA) with a Knoop diamond indenter under a 25 g load for 10 s. Five indentations, 100 μm apart, were made in the center of each block to analyze the SH. SH was measured again after the induction of artificially demineralized lesions (SH1) and after pH-cycling (SH2), at 100 mm from the initial indentations (SH) (Vieira et al., 2005). The percentage of SH recovery was then calculated (% SHR = [{SH2 − SH1}/{SH − SH1}] × 100). For cross-sectional hardness measurements, the blocks were sectioned at the center and one of the halves was embedded in acrylic resin and gradually polished. A sequence of 14 indentations was created at 5, 10, 15, 20, 25, 30, 40, 50, 70, 90, 110, 130, 220, and 330 μm from the enamel surface, in the central region of the blocks, using a Micromet 5114 hardness tester (Buehler Lake Bluff, IL, USA) with a Knoop diamond indenter under a 5 g load, for 10 s. Integrated hardness (KHN × μm) for the lesion into sound enamel was calculated by the trapezoidal rule (GraphPad Prism, version 3.02) and subtracted from the integrated hardness for sound enamel to obtain the integrated area of the subsurface regions in enamel, which was named integrated loss of subsurface hardness (ΔKHN; KHN × μm) (Danelon et al., 2013).

2.6. Analysis of loosely-bound fluoride (CaF_2) on enamel

The concentration of loosely-bound fluoride (CaF_2) on enamel was analyzed after application of gels (CaF_2 formed) and after pH cycling (CaF_2 retained). A digital caliper (Mitutoyo CD-15B, Mitutoyo Corporation, Japan) was used to measure the surface area of the enamel blocks (n = 120) (Akabane et al., 2018). Assessment of loosely bound fluoride (alkali-soluble fluoride – CaF_2 formed and retained) was performed following the methodology of Caslavska, Moreno, and Brudevold (1975). The surface of each specimen, except for the treated surface, was coated with wax. Then, blocks were immersed in 0.5 mL of Potassium hydroxide 1.0 mol/L solution for 24 h under constant agitation. The solution was neutralized and buffered with 0.5 mL of TISAB II modified Hydrochloric acid. Fluoride content was determined using an ion-specific electrode 9409BN (Thermo Scientific, Beverly, MA, USA) and microelectrode reference (Analyser, São Paulo, Brazil) coupled to an ion analyzer (Orion 720A$^+$, Thermo Scientific, Beverly, MA, USA) previously calibrated with standards 4.00–64.00 ppm F (100 ppm F, Orion 940907) that were used for the readings. The data obtained in mV were converted to μg F/cm^2 using Microsoft Excel.

2.7. Analysis of firmly-bound fluoride (FA) in enamel

Blocks measuring 2 mm × 2 mm (n = 120) were obtained from half of the longitudinally sectioned blocks and fixed to a mandrel coupled to a modified microscope with a micrometer (Micrometer 733 MEXFLZ-50, Starret, Athol, MA, USA) to measure enamel wear. Self-adhesive polishing discs (diameter, 13 mm) and 400-grit silicon carbide (Buehler) were fixed to the bottom of a polystyrene crystal tube (J-10; Injeplast, Sao Paulo, SP, Brazil). One layer of 50.0 ± 0.03 μm each was removed from the enamel blocks by grinding the enamel surface against the polishing discs, in circular movements. The vials with the enamel