Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/jden

Short communication

In situ evaluation of low-fluoride toothpastes associated to calcium glycerophosphate on enamel remineralization

A.C.S.F. Zaze, A.P. Dias, J.G. Amaral, M.L. Miyasaki, K.T. Sassaki, A.C.B. Delbem *

Department of Pediatric Dentistry and Public Health, Araçatuba Dental School, UNESP-Univ. Estadual Paulista, R. José Bonifácio 1193, CEP 16011-050, Araçatuba, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form
28 August 2014
Accepted 1 September 2014

Keywords:
Toothpastes
Fluorides
Glycerophosphates
Tooth remineralization
Dental enamel

ABSTRACT

Objectives: This study aimed to evaluate the effect of low-fluoride toothpastes with calcium glycerophosphate (CaGP) on enamel remineralization in situ.
Methods: Volunteers ($n = 10$) wore palatal devices holding four bovine enamel blocks. The treatments involved 5 experimental phases of 3 days each according to the following toothpastes: placebo, 500 ppm F (500 NaF), 500 ppm F with 0.25% CaGP (500 NaF CaGP), 500 ppm F with 0.25% CaGP (500 MFP CaGP) and 1100 ppm F (1100; positive control). After this experimental period, the fluoride, calcium, and phosphorus ion concentrations from enamel were determined. Surface and cross-sectional hardness were also performed. Data were analysed by 1-way ANOVA, Student–Newman–Keuls' test and by Pearson's correlation.
Results: The addition of 0.25% CaGP improved the remineralization potential of low-fluoride toothpastes and the NaF as source of fluoride yielded the best results ($p < 0.001$) as evidenced by the hardness analysis. The 1100 ppm F toothpaste provided higher presence of fluoride in the enamel after remineralization ($p < 0.001$). The addition of CaGP to the NaF and MFP toothpastes led to similar calcium concentration in the enamel as the observed with the positive control ($p = 0.054$).
Conclusions: Toothpastes with 500 ppm F (NaF or MFP) and CaGP showed similar remineralization potential than 1100 ppm F toothpaste.
Clinical significance: Toothpastes containing 500 ppm F associated to CaGP, with both fluoride source (NaF or MFP), showed a potential of remineralization similar to commercial toothpaste. Although there is a need for confirmation in the clinical setting, these results point to an alternative for improving the risk-benefit relationship between fluorosis and dental caries in small children.

* *Corresponding author*. Tel.: +55 18 3636 3314; fax: +55 18 3636 3332.
E-mail addresses: adelbem@foa.unesp.br, adelbem@pq.cnpq.br (A.C.B. Delbem).
http://dx.doi.org/10.1016/j.jdent.2014.09.001
0300-5712/

1. Introduction

Fluoride (F) is the most important agent for preventing dental caries. However, its widespread ingestion is associated with dental fluorosis.[1] One possible method to reduce F ingestion by young children is the reduction of this ion concentration in toothpastes. Nevertheless, toothpastes with low F concentration have shown to be less effective than 1100 ppm F formulations.[2]

As an alternative, studies have been demonstrated that the addition of organic and inorganic phosphates can increase the effectiveness of low F concentration toothpastes.[3–7] Combined to this, the risk of fluoride intake in children using low-fluoride toothpaste associated to organic or inorganic phosphate is very low when compared to 1100 ppm F toothpaste.[8] Among phosphates that have been studied, calcium glycerophosphate (CaGP), which is a source of calcium and phosphate, has demonstrated anti-cariogenic properties. A previous in vitro study[6] demonstrated that the addition of 0.25% of CaGP to low-fluoride toothpastes (500 ppm; NaF) achieved the same anti-caries efficacy than 1100 ppm F toothpastes. The same low-fluoride toothpaste was evaluated in an in situ study on enamel demineralization[5], confirming the results of the in vitro study. However, there is an unmet need to assess the ability of this formulation to activate the remineralization process, since the most important toothpastes properties are the ability of reduce demineralization and increase remineralization process, especially in populations with high caries prevalence.

Despite the association CaGP/NaF demonstrates anti-caries effect, there is a concern regarding the stability of the formulation when combining a source of calcium and sodium fluoride. Hence, CaGP (0.13%) always was associated to monofluorophosphate (MFP; 1000–1500 ppm F).[9–11] However none conclusive anticaries effect was obtained from that association. Thus, this study aimed to evaluate in situ whether the addition of 0.25% CaGP to low-fluoride toothpastes (500 ppm F) with different sources of fluoride (NaF and MFP) would provide similar remineralization potential as a standard toothpaste (1100 ppm F). The null hypothesis was that low-fluoride toothpastes associated to CaGP would lead to same anticaries effect when compared to the standard F toothpaste without CaGP.

2. Materials and methods

2.1. Formulation of experimental toothpastes

Experimental toothpastes were prepared using carboxymethylcellulose, sodium methyl-p-hydroxybenzoate, sodium saccharin, peppermint oil, glycerol, hydrated silica (Tixosil 73, 15%), sodium lauryl sulfate, and water. The fluoride concentrations (NaF – Merck, Germany), in the experimental toothpastes were 0, 500, or 1100 ppm F. The CaGP (dl, 50% α- and 50% β-isomer) concentration in the experimental toothpaste with 500 ppm F (NaF – Merck, Germany and MFP – Sigma Aldrich, USA) was 0.25%. A toothpaste without fluoride or CaGP (placebo) was prepared as a negative control. Total, ionizable and ionic fluoride concentrations were checked using a specific electrode (9609 BN) connected with an ion analyzer (Orion 720 A^{plus}).[12]

2.2. Enamel blocks preparation

Enamel blocks ($n = 200$) measuring 4 mm × 4 mm were obtained from bovine incisors previously stored in 2% formaldehyde solution (pH 7.0) for one month. The enamel blocks were polished with carbide paper (600, 800, and 1200 grit) and diamond abrasives on a polishing cloth to remove ~120 μm of the outer surface. They were selected through surface hardness (SHi) using a microhardness tester (HMV-2000) coupled with CAMS-WIN software with a Knoop diamond indenter under a 25-g load for 10 s^3 and randomly categorised (330.0–370.0 KHN; $p = 0.112$) into five groups of 40 teeth each. Ensuing, the enamel blocks had the surface coated with acid-resistant varnish, except enamel (16 mm^2). Subsurface enamel demineralization was produced by immersing each enamel block in a solution with 1.3 mmol/l Ca, 0.78 mmol/l P in 0.05 mol/l acetate buffer, pH 5.0; 0.03–0.04 μg F/ml (2 ml/mm^2); for 16 h at 37 °C.[13,14] Post-demineralization surface hardness (SHd) was measured through five indentations spaced 100 μm from each other and from the SHi and blocks that presented hardness values between 59.0 e 92.0 KHN ($p = 0.986$) were selected.

2.3. Intraoral procedures

This was a crossover, double blind study, previously approved by the local Human Ethical Committee (protocol#2835/08). Seventeen young adults that present good general and oral health and normal salivary flow rate were randomly selected with no gender distinction. They were living in an area with fluoridated drinking water (0.7 mg F/L). Exclusion criteria included the use of any medicine likely to interfere with salivary secretion, use of fixed or removable orthodontic appliances, pregnancy or breastfeeding, smoking, or presence of any systemic illness. Volunteers who showed average mineral gain of approximately 10–20% using placebo dentifrice (3-day experimental regimen) were selected ($n = 10$). After consent, each volunteer ($n = 10$) was provided with an acrylic palatal device to be worn 24 h/day, containing 4 bovine enamel blocks (4 mm × 4 mm × 2 mm). The treatments involved 5 experimental phases of 3 days each according to the following toothpastes: placebo, 500 ppm F (500 NaF), 500 ppm F with 0.25% CaGP (500 NaF CaGP), 500 ppm F with 0.25% CaGP (500 MFP CaGP) and 1100 ppm F (1100; positive control). The treatments were performed during the volunteers' habitual oral hygiene routine. During the experimental period the volunteers brushed their natural teeth for 1 min and 3 times a day (after the main meals) with the appliance in place, using the toothbrush and toothpaste provided to them.[15] The enamel blocks were directly brushed (exposed to the slurry toothpaste by dilution in human saliva of the volunteers during brushing) in order to provide the most similar situation that occur in the oral environment. Volunteers used the non-fluoride toothpaste during the 7-day pre-experimental and 7-day washout periods. Diet restrictions were not imposed on the volunteers, but they were instructed to remove the