Journal of Dentistry 43 (2015) 1249–1254

Contents lists available at ScienceDirect

Journal of Dentistry

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

Synergistic effect of fluoride and sodium hexametaphosphate in toothpaste on enamel demineralization *in situ*

Danielle Mendes da Camara, Juliano Pelim Pessan, Tamires Melo Francati, José Antonio Santos Souza, Marcelle Danelon, Alberto Carlos Botazzo Delbem*

Araçatuba Dental School, UNESP—Univ. Estadual Paulista, Department of Pediatric Dentistry and Public Health, R. José Bonifácio 1193, 16015-050 Araçatuba, SP, Brazil

ARTICLE INFO

Article history:
Received 29 April 2015
Received in revised form 15 July 2015
Accepted 15 August 2015

Keywords:
Dentifrices
Dental Enamel
Fluorides
Polyphosphates
Demineralization

ABSTRACT

Objective: To evaluate the effect of a fluoride dentifrice containing sodium hexametaphosphate (HMP) on enamel demineralization *in situ*.
Methods: This double-blind and cross-over study consisted of 3 phases (7 days each) in which 12 volunteers wore intraoral appliances containing four enamel bovine blocks. Specimens were treated (3×/day) with placebo (no F or HMP), 1100 ppm F (1100F) and 1100F plus HMP1% (1100F-HMP1%) toothpastes, and the cariogenic challenge was performed using a 30% sucrose solution (6×/day). Final surface hardness, the percentage of surface hardness loss (%SH), the integrated loss of subsurface hardness (ΔKHN), as well as enamel calcium (Ca), phosphorus (P) and firmly-bound fluoride (F) were determined. Also, biofilm formed on the blocks were analyzed for F, Ca, P and insoluble extracellular polysaccharide (EPS) concentrations. Data were submitted 1-way ANOVA, followed by Student–Newman–Keuls' test ($p < 0.05$).
Results: 1100F-HMP1% promoted the lowest %SH and ΔKHN among all groups ($p < 0.001$). The addition of HMP1% to 1100F did not enhance enamel F uptake, but significantly increased enamel Ca concentrations ($p < 0.001$). Similar EPS concentrations were seen for 1100F-HMP1% and 1100F groups ($p > 0.05$). All the groups were supersaturated with respect to HA. However, only 1100F-HMP1% group was supersaturated with respect to CaF_2 ($p < 0.05$). The ionic activities of F^-, CaF^+ and HF^0 for the 1100F-HMP1% group were the highest among all groups ($p < 0.001$).
Conclusion: The addition of HMP1% to a conventional toothpaste significantly reduces enamel demineralization *in situ* when compared to 1100F.
Clinical relevance: This dentifrice could be a viable alternative to patients at high risk of caries.

1. Introduction

Dental caries is a multi-factorial disease with a complex etiology, whose root cause is the bacterial production of acids from dietary sugars at the interface of residual dental biofilm and a susceptible tooth surface [1,2]. The prevention and treatment of early caries lesions, especially in patients at high risk, is a constant challenge in dentistry. In this sense, efforts have been directed to search advances in technologies to promote remineralization of early caries lesions, as well as to reverse the caries process at the earliest possible stage [3].

The use of fluoride dentifrice is regarded as the most effective preventive public health measure to prevent dental caries [4], but conventional dentifrices (1000–1100 ppm F) have a limited effect for high risk individuals, especially those with high biofilm levels and frequent sugar intake [3]. The addition of organic and inorganic polyphosphate salts to fluoridated products for topical use has been shown to be an effective alternative to increase their efficacy against dental caries, both *in vitro* and *in situ* [5–7].

Sodium hexametaphosphate (HMP) is a cyclic inorganic phosphate widely used in the food industry as an antimicrobial agent owing to its ability to increase the permeability of the bacterial outer membrane [8]. HMP was shown to have an inhibitory effect on biofilm formation in hamsters with a high sucrose diet [9]. Also, previous studies have shown that inorganic polyphosphates have anti-caries activity that is related to their interaction with enamel [10].

Da Camara et al. [6] demonstrated *in vitro* that HMP has synergistic effect with fluoride when combined at an appropriate HMP/F ratio, leading to an increase in the anti-caries effect of a

* Corresponding author. Fax: +55 18 3636 3332.
E-mail address: adelbem@foa.unesp.br (A.C.B. Delbem).

http://dx.doi.org/10.1016/j.jdent.2015.08.007
0300-5712/

low-fluoride dentifrice (250 ppm F) to levels similar to those seen for a conventional formulation (1100 ppm F). Given that dentifrices containing 1100 ppm F are the most widely used by the population, it would be interesting to assess the association between fluoride and HMP in these formulations, in order to verify if such combination would further enhance their protective effect against enamel demineralization.

Therefore, the aim of this *in situ* study was to evaluate the effect of HMP added to a dentifrice containing 1100 ppm F on bovine enamel demineralization *in situ*, as well as its effects on the mineral composition of enamel and biofilm. The null hypothesis was that a dentifrice containing HMP presents a similar anticaries effect as its counterpart without HMP.

2. Materials and methods

This study was previously approved by the Human Ethical Committee from Araçatuba Dental School, São Paulo State University, Brazil (CAAE: 30361414.0.0000.5420) and all participants read and signed informed consent statements prior to study initiation.

2.1. Experimental design

This double-blind and cross-over study was performed in 3 phases of 7 days each. A sample size of twelve volunteers was based in previous study [11] considering primary outcomes from surface and cross-sectional hardness analysis, the mean difference among the groups (30 and 1300, respectively), standard deviation (20 and 9000, respectively), an α-error of 5% and a β-error of 20% (SigmaPlot, version 12.0). Volunteers ($n = 12$; female = 2 and male = 10; dental students) 20–30 years old, who were in good general and oral health [12], presented normal salivary flow [13], and did not violate the exclusion criteria (use of any form of medication likely to interfere with salivary secretion, use of fixed or removable orthodontic appliances, pregnancy or breastfeeding, smoking, or systemic illness), were included in the study. They wore acrylic palatal appliances with sound bovine enamel blocks (4 mm × 4 mm, $n = 144$) previously polished and selected using surface hardness test (Baseline 360 up to 380 KHN; $p = 0.991$) and randomly allocated into the groups. A screening *in vitro* study was conducted (data not published) using a pH cycling model [14], in which HMP concentrations of 0.5, 1.0 e 2.0% were added to a conventional dentifrice; the best inhibiting effect on enamel demineralization was seen for HMP at 1%. The specimens were allocated to 3 treatments: without fluoride and HMP (placebo, negative control), 1100 ppm F (1100F, positive control) and 1100F combined with HMP1% (1100F-HMP1%). After each experimental phase, the biofilm formed *in situ* was collected for analysis of fluoride (F), calcium (Ca), phosphorus (P) and insoluble extracellular polysaccharide (EPS). In the enamel blocks, surface and cross-sectional hardness as well as F, Ca, and P content were determined. The main aspects of the study protocol are summarized in Fig. 1.

2.2. Dentifrice formulation

The experimental dentifrices were prepared with the following ingredients: carboxymethylcellulose, sodium methyl-*p*-hydroxybenzoate, sodium saccharin, peppermint oil, glycerol, hydrated silica, sodium lauryl sulfate, and water [5]. The fluoride (NaF, Merck, Darmstadt, Germany) concentration in the experimental dentifrice was 1100 ppm F to which HMP1% (Aldrich Chemistry, CAS 68915-31-1, United Kingdom) was added. To compare and validate the results, the following dentifrices were also manufactured: without fluoride and HMP (placebo) and 1100 ppm F with the same formulation as described previously. Fluoride concentrations in the toothpastes were determined using an ion-specific electrode (9409 BN) connected with an ion analyzer (Orion 720 A^{plus}), previously calibrated with 5 standards (0.125, 0.25, 0.5, 1.0 and 2.0 mg F/mL) [15].

2.3. Enamel blocks and palatal appliance preparation

A total of 144 enamel blocks measuring 4 × 4 × 2 mm were obtained from bovine incisors previously stored in 2% formaldehyde solution (pH 7.0) for 1 month [16]. They were sequentially polished and selected by surface hardness test (330.0–380.0 KHN) as previously described [5] (Fig. 1A). Blocks were then randomly allocated into three groups of 48 teeth each [11]. Four enamel blocks were fixed in the palatal device in each phase. A 4.0-mm deep space was created in the appliances, leaving a 1.0-mm space for dental biofilm accumulation on the enamel blocks. They were protected from mechanical disturbance by a plastic mesh attached to the acrylic surface in order to promote dental biofilm formation [11].

2.4. Intraoral procedures

Fresh 30% sucrose solutions were prepared every 48 h as the cariogenic challenge. The volunteers were instructed to remove the appliances from the oral cavity and drip two drops of this solution (enough to fill the 1.0 mm space) onto each enamel block six times a day at predetermined times (8:00 am, 11:00 am, 2:00 pm, 5:00 pm, 7:00 pm, and 9:00 pm). After dripping, the appliances were left to rest for 5 min before being reinserted in the mouth (Fig. 1B). The appliances were used 24 h a day and treatments with the dentifrices were performed 3 times a day. The volunteers brushed their natural teeth 3 times a day (08:00 am, 13:00 pm, 21:30 pm) during 2 min, with palatal appliance in the oral cavity, allowing the natural saliva/dentifrice slurry to come into contact with the enamel blocks by gently squishing the slurry in the mouth. Following, the devices were removed from the oral cavity and gently rinsed with tap water; volunteers then brushed their natural teeth and rinsed the mouth as usual, returning the devices to the oral cavity immediately afterwards. The volunteers were instructed to remove the palatal appliance before drink or eat. During the 7-day lead in and wash out periods, volunteers brushed their teeth with a toothpaste without fluoride and HMP. The volunteers received all instructions before initiation of the experiment.

2.5. Hardness analysis

Enamel surface hardness was determined before and after each phase in each specimen by using a Shimadzu HMV-2000 microhardness tester (Shimadzu Corp., Kyoto, Japan) under a 25-g load for 10 s. Five initial indentations (SHi, baseline) spaced 100 μm from each other were made in the center of all enamel blocks. After each phase, 5 final indentations (SHf) were made spaced 100 μm from the baseline indentations (Fig. 1D) to calculate the percentage of surface hardness loss (%SH = [(SHf − SHi)/SHi] × 100) [5].

Blocks were then cross-sectioned and half of each block was embedded in acrylic resin and gradually polished (Fig. 1E). One sequence of 14 indentations at different distances (5, 10, 15, 20, 25, 30, 40, 50, 70, 90, 110, 130, 220, and 330 μm) were made in the surface of the enamel in the central region Micromet 5114 hardness tester (Buehler, Lake Bluff, IL, USA) and the software program Buehler OmniMet (Buehler) with a Knoop diamond indenter under a 5-g load for 10 s [17]. 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