




In Situ Effect of Intra-Oral Application of Pastes Containing CPP-ACP or CPP-ACPF Against Initial Enamel Erosion

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Abstract

Objective: To evaluate in situ the effect of toothpastes containing casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) and casein phosphopeptide-amorphous calcium phosphate associated to fluoride (CPP-ACPF) on initial erosion prevention. **Material and Methods:** Bovine enamel blocks (n = 192) were randomly assigned into 4 phases according to the baseline surface hardness: GI: CPP-ACP Paste (MI Paste™), GII: CPP-ACPF Paste (MI Paste Plus™), GIII: Fluoridated paste and GIV: Placebo Paste. In each of the 4 crossover phases, twelve volunteers wore intraoral palatal appliances containing 4 enamel blocks for 2 hours, then the tested treatments were applied intraorally (3 min) and the appliance was maintained in the mouth for another 3 hours. After, the appliances were removed and immersed in hydrochloric acid (0.01 M, pH 2.3) for 30 seconds to promote erosive demineralization. The final surface hardness was evaluated and percentage of surface hardness loss was calculated. The data were analyzed by ANOVA and Tukey's test ($\alpha = 5\%$). **Results:** The application of CPP-ACP paste, independent of fluoride content, resulted in significant lower enamel hardness loss (GI: 9.26% \pm 3.48 and GII: 9.14% \pm 1.73) compared to NaF (GIII: 15.5% \pm 3.94) and placebo (GIV: 16.7% \pm 4.07) pastes, which did not show difference between them. **Conclusion:** The CPP-ACP pastes were able to reduce initial erosive demineralization in relation to fluoride and placebo pastes. Nevertheless the formulation of CPP-ACP with fluoride did not provide an additional benefit.

Keywords: Dental Enamel; Tooth Erosion; Caseins; Sodium Fluoride.

Introduction

Erosive tooth wear has been widely studied [1,2] because of its increasing incidence [3] resulting from the modern society lifestyle and substantial changes in eating habits [4,5]. Advanced tooth erosion can affect aesthetics and function and induce hypersensitivity [6]. Therefore, it is essential to focus on the initial phases of the erosive process [5,7], by establishing appropriate measures of erosion prevention and control [1].

The casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been considered a possible alternative to fluoride in the prevention of dental erosion [8]. CPP-ACP nanocomplexes have binding affinity with the proteins of the salivary acquired pellicle, reinforcing its natural protective capacity [9]. In addition, CPP-ACP can promote mineral precipitation on eroded surfaces through the bioavailability of calcium and phosphate ions [10].

Nonetheless, the main objective of dental erosion treatments should be the protection of the surface against demineralization rather than its remineralization [11]. Although some studies have already evaluated the effect of CPP-ACP pastes before the erosive challenge [12-15], their controversial results, mainly due to the lack of methodological standardization, do not allow a conclusion on the inhibition of demineralization [16]. Moreover, most of those studies were conducted in vitro [12-14,17,18], which disconsider several factors from the oral cavity that influence the erosive dynamics, such as saliva and enamel acquired pellicle [7].

The effect of CPP-ACP and CPP-ACPF on dental erosion has been assessed previously [19,20]; however, none of the studies evaluated the preventive effect of the pastes, once the pastes were applied after the erosive challenge. Moreover, in situ studies are scarce on the preventive effect of the CPP-ACP paste on initial erosion of enamel [15]. Also important is the lack of comparisons between the CPP-ACP paste and a conventional fluoride toothpaste for daily oral hygiene.

Therefore, this in situ study was designed to evaluate the preventive effect of the CPP-ACP paste, supplemented or not with fluoride, and compare to sodium fluoride paste and placebo against a short-term acidic exposure. The null hypothesis tested was that there is no difference in inhibition of initial erosive demineralization of enamel with the use of CPP-ACP, CPP-ACPF, and the other pastes.

Material and Methods

Experimental Design

The study was conducted with a double-blind and randomized four-way crossover protocol, and paired phases of 5 hours, with an interval of one week between them. The factor under evaluation was the preventive treatment against dental erosion at four levels: GI - CPP-ACP Paste, MI Paste™ (GC America Inc., Alsip, IL, USA); GII - CPP-ACP + NaF 900 ppm Paste, MI Paste Plus™ (GC America Inc., Alsip, IL, USA); GIII - Fluoridated paste, NaF 900 ppm (Dilecta® Farmácia de Manipulação & Homeopatia, João Pessoa, PB, Brazil); and GIV - Placebo Paste (Dilecta® Farmácia de Manipulação & Homeopatia, João Pessoa, PB, Brazil). Bovine enamel blocks (n = 192) were

randomly assigned to 12 volunteers who wore acrylic palatal appliances containing 4 blocks per phase for intra-oral application of the tested pastes. The CPP-ACP pastes are not toothpastes but instead they are indicated for topical application. Therefore, all studied pastes were applied by the volunteers with a finger. Immediately after usage, the palatal appliances were immersed in hydrochloric acid solution (0.01 M, pH 2.3) for 30 seconds to provide a short-term acidic challenge. The percentage of surface hardness loss was quantified.

Tooth Selection and Sample Preparation

Three hundred sixty-five bovine enamel blocks (4 x 4 x 3 mm) were prepared from bovine incisors recently extracted and kept in 0.1% thymol solution at pH 7.0. The enamel blocks were cut using a cutting machine (ISOMET Low Speed Saw, Buehler Corp., Binghamton, NY, USA) and an aluminum oxide disk (Bosch 2, AS 60T INOX BF, 115 x 1 x 22.23 mm, Gerlingen, Germany). A digital pachymeter (Mitutoyo America Co., Illinois, USA) was used to measure the enamel blocks dimensions.

The surface of the enamel blocks was ground flat with water-cooled sandpaper discs 600, 1200, 1500 and 2000 grades (3M Company, Minnesota, USA) and polished with felt paper wet by diamond spray (Buehler Corp., Binghamton, NY, USA). Broken or cracked enamel blocks were discarded and the initial surface hardness (SHi) was determined by performing five indentations at distances of 100 µm from each other on the center of the blocks (Vickers Diamond point, 100 g, 15 s, Microhardness Tester FM-700, Future-Tech Corp., Fujisaki, Kawasaki-ku, Japan). One hundred and ninety-two enamel blocks with average initial surface hardness ranging from 324 to 379 HV were selected and randomly assigned to the volunteers and the study groups. The position of the enamel blocks on the right or left side of the appliance was also randomly determined. The enamel blocks were sterilized by exposure to ethylene oxide gas previous to the in situ phases [21,22].

Volunteers and in Situ Experiment

Sample size calculation was based on a pilot in situ study. A minimum detectable difference in hardness loss between groups of 6%, an estimated standard deviation of 4%, a statistical power of 80%, and level of significance of 5% were considered. A sample size of 11 volunteers was estimated; however, for easier allocation on volunteers into groups of the cross-over design, 12 were selected.

The inclusion criteria for volunteers were stimulated salivary flow rate > 1 mL/min, non-stimulated physiological salivary flow rate > 0.25 mL/min, and adequate oral health, with no caries lesions, tooth wear or gingivitis/periodontitis. The exclusion criteria were systemic illness, pregnancy or breastfeeding, orthodontic treatment, use of fluoride compounds in the last 2 months, smokers, and users of acidic medications. Eight females and 4 male, aged 22–33 years were thus selected and took part in the study after giving informed written consent.

The intraoral palatal appliances were made with acrylic resin on a plaster model of the upper arch of each volunteer; a new appliance was made for each phase of the study [21,23]. Acrylic

appliances had two vertical rows, one on the right and the other on the left side, with one cavity ($12 \times 6 \times 3$ mm) for the fixation of two blocks. Blocks were fixed with wax and carefully leveled to the appliance surface to avoid dental plaque accumulation. An orthodontic wire was fixed with resin above the enamel blocks but without touching their surfaces to avoid tongue contact [22,23].

Seven days prior to and during the experimental period, the volunteers brushed their teeth with a standardized toothbrush (Oral B, Classic, Acumen Houseware Industry Co. Ltd, Nanning, China), fluoride toothpaste (Colgate Total 12[®] Clean Mint, 1,450 ppm F, Colgate-Palmolive Industrial Ltda., São Paulo, SP, Brazil) and dental floss (Cosmed Ind. Cosméticos e Medicamentos S/A, Barueri, SP, Brazil). The volunteers were asked not to use any other fluoride product [21,22].

At each phase of the in situ experiment, the volunteers received the intraoral appliances wrapped in humid gauze in a plastic case, and the test paste packed in a color-coded plastic packaging by a researcher not involved with data collection. The participants received written instructions for the in situ phases, which were verbally reinforced.

In each phase, volunteers brushed their teeth with fluoride toothpaste 1 h prior to insertion of the intraoral appliances (8 am), aiming to standardize the amount of residual fluoride present in the mouth. They were instructed to wear the appliances for 2 hours to allow the formation of acquired pellicle [23].

Thereafter, volunteers applied the provided paste with their finger for 3 minutes, following manufacturer instructions. Then, the participants were instructed to spit out the remaining paste without rinsing the mouth. The appliances were used for another 3 hours, during which the volunteers did not eat or drink. Then, the appliances were removed and wrapped with gauze moistened with mineral water (Crystal Águas do Nordeste Ltda, 0.04 mg/L F, pH 6.21, Maceió, Brazil), packed in a plastic case and collected by the researcher to perform the erosive challenge.

The erosive challenge was performed by the immersion of the appliances in 150 mL of hydrochloric acid solution (Vetec Química Fina Ltda., Rio de Janeiro, Brazil, 0.01 M, pH 2.3) for 30 seconds, under agitation, followed by washing with deionized water, based on a previous study that evaluated different erosion protocols [23].

Surface Hardness Measurement (SHf) and Percentage of Surface Hardness Loss (%SHL)

The final surface hardness was determined by performing five indentations at 100 μ m distances from each other and 100 μ m from the initial indentations, with the same measurement parameters as described above. The mean values were obtained from the initial and final surface hardness and were used to calculate the percentage of surface hardness loss: $\%SHL = [(SH_i - SH_f) / (SH_i)] \times 100$, where %SHL is the percentage of surface hardness loss, SH_i is the initial surface hardness and SH_f is the final surface hardness.

Statistical Analysis

Statistical analysis was performed with SigmaPlot version 12.3 (2011 Systat Software GmbH, Erkrath, Germany). The assumption of equality of variances was met and Shapiro-Wilk test was used to check the normality of the data ($p > 0.05$). One way repeated measures ANOVA for a crossover study was used to compare the effects of treatments, study period, and study sequence, and their interactions on the enamel hardness loss. The models also included a random effect to account for multiple measurements within a subject. Tukey's test was performed for multiple comparisons. The level of significance was set at 5%.

Ethical Aspects

This in situ study was approved by the local Institutional Ethics Committee (Protocol No. 57770916.4.0000.5187) and followed the guidelines of the Declaration of Helsinki.

Results

All twelve volunteers completed the in situ protocol and no side effects were reported. Table 1 shows the mean of the initial and final hardness values and the percentage of hardness loss of the studied phases. The first column shows that in all experimental phases the enamel blocks presented similar initial hardness. The second column gives the surface hardness after treatments and erosive challenge. Data from the first and second columns were used to calculate the surface hardness loss, which is presented in the third column.

Table 1. Mean and standard deviation of initial (SH_i) and final enamel surface hardness (SH_f) and the percentage of surface hardness loss (%SHL) for the experimental phases (n=12).

Experimental Phases	SH _i - HV	SH _f - HV	%SHL (± SD)
P I (CPP-ACP)	355.58 (± 15.44)	322.58 (± 21.15)	9.26 (± 3.48) ^a
P II (CPP-ACPF)	355.03 (± 12.99)	322.40 (± 15.52)	9.14 (± 1.73) ^a
P III (Sodium Fluoride)	355.24 (± 13.60)	299.97 (± 20.24)	15.5 (± 3.94) ^b
P IV (Placebo)	354.96 (± 15.51)	295.58 (± 19.57)	16.7 (± 4.07) ^b

*Phases whose means are followed by distinct superscript letters differ significantly (ANOVA models and Tukey's test, $p < 0.005$).

There was a significant difference among groups. The CPP-ACP and CPP-ACPF pastes contributed to lower enamel hardness loss compared to sodium fluoride and placebo pastes ($p < 0.05$), with no difference between the last two groups.

Discussion

The diagnosis and early intervention on initial erosive lesions allows the prevention of additional damages due to erosive tooth wear [2]. The present study evaluated the in situ effect of pastes containing the CPP-ACP nanocomplex, supplemented or not with sodium fluoride, in relation to a paste with the same type and concentration of fluoride and a placebo paste. The null hypothesis was rejected, since the use of the CPP-ACP and CPP-ACPF pastes provided less surface hardness loss compared to the fluoridated and placebo pastes.

Surface hardness loss was used to measure the effect of CPP-ACP pastes prior to a short-term acidic exposure since the first stage of the erosive lesion is characterized by the decrease in mechanical resistance and hardness of the enamel superficial layer [24]. The baseline indentation impressions were visible after the erosive challenge, suggesting the absence of erosive enamel loss, that is, the final surface (after treatment and erosion) and the initial surface were almost the same [21,25].

Initial erosion lesions were induced by hydrochloric acid exposure, which has been used in several *in situ* studies [9,23,25,26] to simulate the acidity of the gastric juice, often an intrinsic cause for the development of dental erosion [27]. The erosive challenge was performed *ex vivo* due to ethical restrictions and to guarantee greater control and standardization of the demineralization. The enamel blocks were removed from the volunteers' mouth and immediately subjected to a short-term acidic exposure because a prolonged maintenance of the appliance outside the oral cavity could modify some proteins of the acquired pellicle [22]. Caution was taken also to avoid touching the surface of the blocks, since the integrity of the acquired pellicle is important to the CPP-ACP mechanism of action [9]. It is important to point out that the four pastes evaluated in this study were of the same flavor (mint) to provide a similar gustatory stimulus and hence the similar salivary stimulations.

Despite the effectiveness of stannous fluoride in the treatment and prevention of dental erosion [7], the sodium fluoride paste was selected for this study based on its popularity, since fluorides are the most common active ingredients in oral prophylactic products [9]. In addition, we used the same type and concentration of fluoride present in the CPP-ACPF paste to better understand the effect of each component of the product. Nonetheless, no significant difference was observed in the protection against erosive demineralization offered by the fluoridated paste compared to placebo. In addition, there was no additional effect observed for CPP-ACPF compared to CPP-ACP.

The protective action of sodium fluoride on dental erosion is presumably due to the formation of a calcium fluoride layer on the tooth surface, protecting the dental structure from the erosive agents [28]. A complete effectiveness of the calcium fluoride layer still depends on a stable and dense structure to constitute a physical barrier [29]. Besides being very thin [30], the layer deposited by sodium fluoride use is unstable and fragile [31], not completely covering the dental surface [30]. Consequently, the protection given by the use of sodium fluoride has a short life [11] and is easily solubilized, becoming less effective against acid attacks [28], which explains the limited effect of the fluoridated paste used in this study on the inhibition of erosive demineralization. Since sodium fluoride was used in the CPP-ACPF paste, the absence of the expected additional protection of this paste compared to CPP-ACP is thus justified. However, in long-term erosive protocols CPP-ACPF could provide a further benefit and for this reason, it should be assessed in future research.

In contrast, the results showed that the CPP-ACP pastes, regardless of fluoride supplementation, promoted greater protection against erosive demineralization, since the surface

hardness loss after the erosive challenge was significantly lower in the groups that used those pastes compared to fluoridated paste and placebo. The mechanism of action of CPP-ACP nanocomplex is through the modification of proteins and enhancement of the protective capacity of the acquired pellicle [9]. CPP-ACP acts as a physical barrier, reducing the permeability of the acquired pellicle and increasing its tenacity. It also provides calcium and phosphate ions that are dissolved before dental enamel, when subjected to an erosive challenge [9]. Thus, it is hypothesized that a thicker and stronger protective layer is formed on the dental surface, which explains the increased protective effect of CPP-ACP pastes.

The vast majority of previous studies that evaluated the preventive effect of CPP-ACP pastes on dental enamel was performed under strict laboratory conditions [12,13,14,17,18] and the results, therefore, cannot be compared with those of the present study. Despite using an in situ protocol to study the effect of prior application of the CPP-ACP paste, some authors compared it with a potassium nitrate dentifrice, not CPP-ACPF paste, and an erosive cycling model was performed [15], which also hinder comparisons with our results.

The results suggest that the CPP-ACP pastes can hamper erosive demineralization of the enamel, which could benefit patients at risk of dental erosion. However, both the protective effect of CPP-ACP pastes against initial enamel erosion and their ability to prevent erosive tooth wear should be further investigated before generalizing this preventive resource for clinical purposes.

Conclusion

It is suggested that the use of dental paste containing CPP-ACP, supplemented or not with sodium fluoride, before an erosive challenge offers a preventive effect against initial erosion. Additional studies are necessary to evaluate the effect of this material in the prevention of erosive tooth wear, as well as to demonstrate its clinical efficacy.

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Conflict of Interest: The authors declare no conflicts of interest.

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