Article published online: 2021-11-01

ORIGINAL ARTICLE

Single application of casein phosphopeptide-amorphous calcium phosphate paste-based paste prevents *in vitro* erosive wear

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ABSTRACT

Aim: The purpose of this study was to evaluate, *in vitro*, the effect of a single application of a casein phosphopeptide-amorphous calcium phosphate paste (CPP-ACP) in comparison with a fluoride varnish, in enamel of bovine teeth, after erosive challenge using cola soft drink. **Materials and Methods:** Forty-five enamel specimens were obtained from bovine teeth, selected according to the surface hardness and randomly divided into three groups: G1 – Fluoride varnish, G2 – CPP-ACP-based paste, and G3 – Control, without any treatment. The enamel specimens were treated using each group material and subjected to three daily erosive challenges by immersion of the specimens in cola soft drink for 5 min, during 5 days. Between the erosive cycles, the enamel specimens were immersed in artificial saliva. The initial profilometry (prechallenge) and the final profilometry (postchallenge) were used to evaluate the enamel loss. **Statistical Analysis:** Data were analyzed using ANOVA and Tukey's Test ($P < 0.05$). **Results:** The single application of CPP-ACP (G2: 4.17 ± 0.77 μm) resulted in less enamel loss compared to control (G3: 4.74 ± 0.52 μm) and higher enamel loss compared to fluoride varnish application (G1: 3.01 ± 0.45 μm) ($P < 0.05$). **Conclusion:** The CPP-ACP paste was effective in reducing enamel wear after erosive cycles, however, the best potential for erosion prevention was observed when using the fluoride varnish.

Key words
Casein phosphopeptide-amorphous calcium phosphate paste, dental erosion, dental wear, Duraphat, *in vitro*

INTRODUCTION

Preventive strategies are important to avoid enamel minerals loss when teeth are subjected to erosive challenges.[1,2] Currently, the most used compound to prevent the enamel erosive wear is fluoride in high concentration or associated with other metallic ions.[2] In general, the fluoride mechanism of action is related to the formation of "CaF_2 like" layer, with or without the presence of metallic ions. This layer might prevent the contact between acid and tooth.[3-7] However, the resistance or the permanency of this layer is limited, being eliminated during high and frequent erosive challenges.[3,4] Therefore, there is a need for alternative products to prevent the dental erosion.

Among these alternatives, products containing calcium and phosphate in the composition have been proposed. The nanocomplexes of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) are peptides derivative from casein, where the ACP is stabilized by CPP, acting

Access this article online	
Quick Response Code:	Website: www.ejgd.org
	DOI: 10.4103/2278-9626.179544

How to cite this article: de Oliveira DS, Santin GC, Honorio HM, Rios D, Gaton PH, da Silva LB, *et al.* Single application of casein phosphopeptide-amorphous calcium phosphate paste-based paste prevents in vitro erosive wear. Eur J Gen Dent 2016;5:69-73.

as a calcium and phosphate reservoir, which can be incorporated in the dental surface. The CPP-ACP promotes an effect of supersaturation of the ions, reducing demineralization and increasing mineralization.[8-12] This mechanism provides anticariogenic properties to CPP-ACP.[13,14]

Regarding dental erosion, the effect of CPP-ACP has not been completely clarified.[1,8,12] In recent studies, the main used form for CPP-ACP is a paste daily applied by the patient. Some *in vitro* studies have shown the decrease in dental erosion with frequent application of this compound.[9-11,15-17] However, patient collaboration when using domestic products is not always correctly performed. There is no data in the literature considering the worst scenario of CPP-ACP use by patients, which is an isolated topic application, like fluoride, varnish professional application. Therefore, the aim of this study was to evaluate, *in vitro*, the effect of the CPP-ACP paste single application in comparison with fluoride varnish, in bovine teeth, undergoing an erosive challenge using a cola drink.

MATERIALS AND METHODS

This study used enamel specimens obtained from bovine incisors crowns. The bovine teeth were extracted from Nellore cattle with an average age of 36 months, after being slaughtered for consumption (Frigorífico Vangélio Mondelli Ltda., Bauru-SP, Brazil). The specimens were cut using two double-faced diamond discs (XLI 2205, 102 mm × 0.3 mm × 12.7 mm; Extec Corp., Enfield, CT, USA) and a stainless steel spacer (7 cm diameter and 4 mm thickness) in between the discs, refrigerated with deionized water, resulting in enamel specimens of 4 × 4 × 3 mm^3. The specimens were planned and polished in a metallographic polishing machine (APL 4, Arotec, Cotia, SP, Brazil) using silicon carbide paper, granulation 320, 600, and 1200 (Extec Corp.).

For the sample selection, the specimens were initially evaluated according to the superficial hardness using a hardness tester (HMV-2000/Shimadzu Corporation Japan– diamond pyramidal penetrator Knoop type, with a static charge of 25 g, for 5 s). Five random indentations were performed to each specimen. The inclusion criteria were sound enamel with superficial hardness between 315 and 380 KHN. The exclusion criteria for enamel specimens were the presence of visible white spot and fracture lines, and hardness value average 10% above or under the total mean value of specimens. Finally, 45 specimens were selected (hardness average: 348.48 ± 17.82) and randomly divided into three groups: (G1) Fluoride varnish application (Duraphat-Colgate® GmbH, Waltrop, Alemanha); (G2) CPP-ACP paste application (GC Tooth Mousse-Recaldent®-GC Corporation, Tokyo, Japan); and (G3) negative control group, no treatment applied.

The evaluation of enamel wear (μm) was measured using a profilometer machine Mahr (Marsurf GD 25, Göttingen, Germany) for initial (prechallenge) and final (postchallenge) evaluations of the enamel. The profilometer machine has a spherical measuring probe tip that goes through the enamel surface in five standard positions, making the initial and final reading at the same place. Before the initial profilometry reading, two lines were made, using scalps, dividing the surface into three areas. The two outer thirds were protected with nail varnish. The superposition of the initial and final graphics was at a standard area to allow wear measurement.

After undergoing the initial profilometry, the specimens were stored in artificial saliva for 24 h to hydration, and after this period, each group was subjected to the proposed treatments.

Group 1 (G1) had the enamel surface covered by a fluoride varnish layer (Duraphat, Colgate-Palmolive GmbH, Waltrop, German) using a microbrush. After the single application, the specimens of this group were immersed in artificial saliva for 4 h, followed by the removal of the fluoride varnish using gaze, simulating the clinical situation of varnish removal by toothbrushing, saliva, tongue, and buccal mucosa a few hours after application.

In Group 2 (G2), a CPP-ACP-based paste (GC Tooth Mousse-Recaldent®, GC Corporation, Tokyo, Japan) was applied by a single application, using a microbrush rubbing on the enamel surface, washed with saliva for approximately 3 min, and then the product excess was removed, simulating its clinical application.

Group 3 (G3), the control group, was subjected to the erosive challenge, without any surface protection treatment.

Following the removal of the varnish layer for Group 1 and application of the CPP-ACP paste on Group 2, the specimens of the three groups were subjected to the erosive challenge, performed during five consecutive days. The 15 specimens of each group were immersed, separately in 600 mL of cola soft drink (Coca-Cola®), for 5 min, 3 times per day. After the immersion, the specimens were washed with distilled water and stored in 1.5 L of artificial saliva (osmotic water 98,542%, sodium benzoate, 0.2%, dehydrated calcium chloride 0.015%, magnesium chloride hexahydrate 0.005%, potassium chloride 0.12%, and carboxymethylcellulose 1%). Between each erosive challenges, the specimens were kept in artificial saliva for at least 4 h.

After the erosive cycles and before the final profilometry reading, the nail varnish was totally removed. The reading at the profilometer was performed conform the

initial reading standards, following the parameters and reading numbers of each specimen. The profile of each reading was obtained and superposed using the software Marsurf XCR 20 (Marsurf GD 25, Göttingen, Germany). Superposition of the profiles allowed the measurement of the difference between initial and final reading, resulting in the numeric data representing the erosive wear suffered by the enamel. All the experimental steps are described in Figure 1.

The obtained data showed normal distribution and homogeneity of variance, verified by the Kolmogorov–Smirnov test and Levene test, respectively. Then, the parametric test of analysis of variance was used for the comparison of the three groups followed by the Tukey's Test for multiple comparisons inter-groups, adopting a significance level of 5%. The data were analyzed using the program Statistical 11.0 (Softonic Internacional S.A., Barcelona, Spain).

RESULTS

The mean of the values obtained after profile superposition were 3.01 ± 0.45 μm, 4.17 ± 0.77 μm, and 4.74 ± 0.52 μm to Groups 1, 2, and 3, respectively.

The single topic application of the CPP-ACP paste was effective to protect the enamel, once the tissue wear was significantly lower when compared to the control group. In the other hand, the CPP-ACP paste effect was not similar to the group in which fluoride varnish was applied ($P < 0.05$), whereas this last, resulted in higher enamel protecting effect facing the erosive challenges.

Figure 2 presents the means of the results among the groups, where the different letters represent statistically significant differences.

DISCUSSION

This study aimed to evaluate the preventive effect of a CPP-ACP paste in comparison with fluoride varnish, both in one single application, over enamel specimens undergoing the erosive challenge. The results showed that the lowest values of dental wear after erosive cycle were obtained in the group treated with fluoride varnish. It is important to highlight that the results obtained using varnish are correlated with the fluorides effect and not by the mechanical protection offered by the varnish, since this had been removed before the erosive challenge.[10] High concentration fluoride, as varnishes have been related with the decrease of erosion in enamel and dentin[18-20] and therefore, routinely indicated.

Although the fluoride products present satisfactory results to minimize tooth wear due to the erosive challenges,[20] new products have been studied, among them, the CPP-ACP. In this study, in the group where the paste containing CPP-ACP was used, less enamel wear was observed than control, which had not preventive therapy. However, its effect was lower when compared to the fluoride varnish. Opposite results had been reported by Carvalho *et al.*[10] when evaluated the effect of fluoride varnish (Duraphat®-Colgate), a CPP-ACP paste (MI Paste Plus®-GC America Inc.) and calcium nanophosphate based paste (Desensibilize Nano P®-FGM) in the prevention of development of enamel erosion. They found no significant difference between control (no treatment)

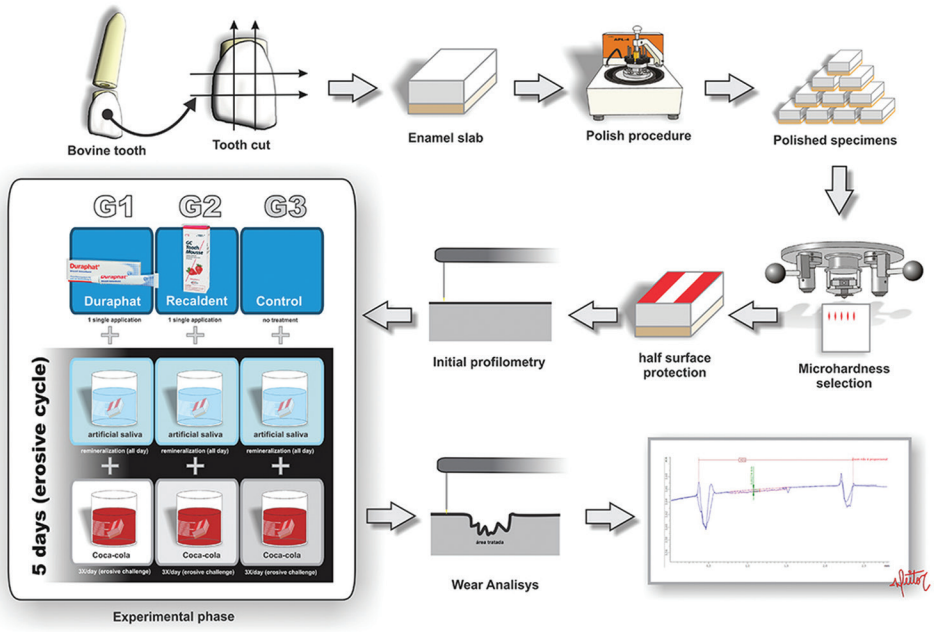

Figure 1: Schematic drawing of the experimental design

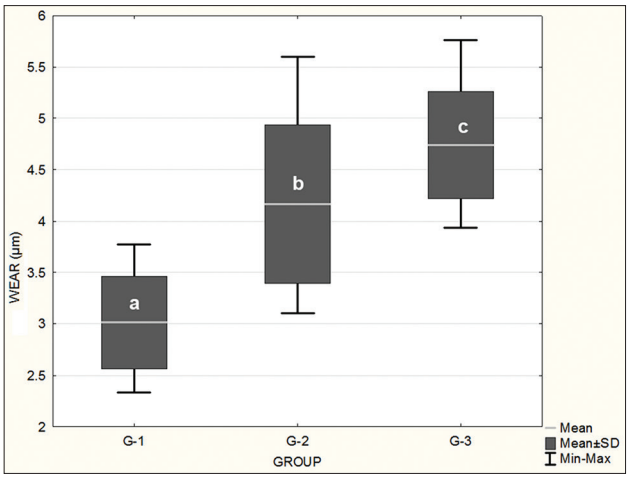

Figure 2: BOX-PLOT graph presenting the mean values, standard deviation, minimum and maximum of the analyzed groups. Different letters indicate the statistically significant difference in the comparison among groups (analysis of variance and Tukey's test; $P < 0.05$)

and varnish, which resulted in the higher loss of hardness than the CPP-ACP paste. It is important to note that in the study described above, the CPP-ACP paste contained fluoride and was applied previously to each erosive challenge, what makes it difficult to assign better results only to CPP-ACP. In addition, the variable response used was the superficial hardness, which is associated to the characteristics of surface mineralization and not to the wear suffered by the enamel. The controversy regarding the effectiveness of CPP-ACP in dental erosion is also found in the literature.[7,11,21,22] Different studies results had shown that CPP-ACP presented efficacy to prevent erosion,[9,11,15,17,23,24] while others studies did not demonstrated such potential.[22,25-27]

The lack of consensus regarding antierosive properties of CPP-ACP can be attributed to the variability in the studies designs.[21] In this study, the product was applied for 3 min, only the excess was removed and also only a single application was performed. The CPP-ACP absence of effects has been related to the lower frequency and short time of application and to the removal of the product by the contact with saliva.[21,22,26] Multiple applications, that would simulate the daily application of the product, could have resulted in an effect more similar to the fluoride varnish. It is also important to highlight that the casein has a low affinity to the enamel after erosive challenges, what can be explained by the fact that this, in acidic conditions, carries a positive charge.[26] However, this characteristic had not affected the results of this study, once the CPP-ACP paste was applied previously to the erosive challenge, when the enamel was sound.

This study design included the analyses of the preventive effect of CPP-ACP and not its therapeutic effect, since that the product was not applied directly to erosive lesions. Therefore, the single application of a CPP-ACP paste showed to be limited, as the fluoride in high concentrations still presents better effect in preventing dental erosion. Possibly, the effect of CPP-ACP is related with the formation of a layer that seals the interprismatic cavities and covers the prisms slowing the attack of acid substances.[15,28] However, according to this study results, this effect was not sustained for 5 days *in vitro*.

Based on the results of this study, a single application of CPP-ACP paste does not overcome the effect of fluoride varnish, being necessary to evaluate multiple *in situ* applications before concluding its potential to the clinical application.

CONCLUSION

Considering the limitations of the present *in vitro* study, our results suggest that a single topic application of a CPP-ACP paste was effective to reduce the dental wear after erosive challenge, although the best potential for the prevention has been encountered after the use of fluoride varnish.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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