



PREVENTIVE EFFECT OF CRANBERRY GEL ON DENTIN SUBMITTED TO EROSION

Joana Regina Dokko¹, Caroline Simões Fávero¹, Ana Claudia Scaraficci¹, Anuradha Prakki², Daniela Rios¹, Heitor M. Honório¹

¹ Department of Pediatric Dentistry, Orthodontics and Public Health, Bauru School of Dentistry - University of São Paulo, Bauru-SP, Brazil
² Department of Clinical Sciences (Restorative), Faculty of Dentistry - University of Toronto, Toronto-ON, Canada

CORRESPONDING AUTHOR: heitorhonorio@usp.br

ABSTRACT

Aims: Considering that Cranberry's components might inhibit dentin metalloproteinases exposed to erosive agents, the aim of this study was to evaluate *in situ* effect of a Cranberry gel application on dentin before an erosive challenge.

Materials and methods: This crossover double-blinded study was performed in 2 phases of 5 days each, with 10 healthy volunteers who wore 2 palatal devices (1 for each phase) with 4 dentin specimens (2 specimens for each group). The groups under study were: First Phase: G1 - Erosive challenge (Coca-cola®) over dentin without any previous treatment (1st negative control group); G2 - Erosive challenge over dentin previously treated with Cranberry gel (test group); and Second Phase: G3 - Erosive challenge over dentin previously treated with a gel without any active principle (2nd negative control group); G4 - Erosive challenge over dentin previously treated with 0.12% Chlorhexidine gel (positive control group). Each device was immersed into the acid beverage, 3 times daily for 5 minutes during 5 days. Profilometry (μm) was used to quantify the dentin wear. Data were analyzed by Repeated Measures Analysis of Variance followed by Fisher's test ($p < 0.05$).

Results: Data (G1: $4.98 \pm 1.36a$; G2: $3.29 \pm 1.16b$; G3: $4.38 \pm 1.19a$; G4: $3.32 \pm 1.55b$) showed no statistical difference between G1 and G3. There was also no difference between G2 and G4. However, G2 and G4 presented lower wear when compared to G1 and G3, and this difference was statistically significant.

Conclusion: The results of this study suggest a significant efficacy of Cranberry gel in preventing wear of dentin subjected to dental erosion.

KEYWORDS: Collagen. Dentin. Erosion. Preventive dentistry. Wear.

INTRODUCTION

Tooth wear promoted by erosive process can be described as lesions characterized by the loss of tooth structure by the action of acids without the involvement of microorganisms, involving intrinsic or extrinsic processes¹⁻⁴ and affects both tooth enamel and dentin.

Unlike enamel, dentin is constituted by 47% of inorganic components (apatite), 33% organic component and 20% water. Among the organic components, 90% consists of Type I collagen and 10% non-collagenous components^{4,5}. The dentin demineralization rate decreases when the amount of degradable collagen

increases, thus the maintenance of collagen hinders the diffusion of acid into the tissue, minimizing erosion. However, the organic matrix can be degraded mechanically and chemically, contributing to the progression of wear of the dentin⁶. This chemical degradation occurs, by the presence of enzymes in dentin called matrix

metalloproteinases (MMPs), which can be activated by the fall in pH below 4.57.

After an erosive challenge, the drop in pH in addition to demineralization of dentin that exposes collagen, active fibrils MMPs that degrade this demineralized organic matrix. All this enables the progression of loss of dentin tissue. Thus, the application of MMP inhibitors could reduce this loss of dentin for subsequent erosive challenges as the organic matrix function as a protective layer which hinders the diffusion of the acid, reducing the progression of the erosion⁸.

In this sense, the maintenance of organic matrix may decrease the erosive progression in future erosive challenges and this fact can be obtained by using MMPs inhibitors. This strategy has shown good results, with the use of Chlorhexidine and Green Tea as inhibitors of MMPs, acting in reducing the degradation of the collagen matrix in both *in vivo*⁹ and *in vitro*¹⁰ studies.

In this sense, alternative methods that may have an inhibitory action of MMPs are well accepted. In medical research, on dental caries and periodontal disease, some benefits related to Cranberry's juice polyphenols (*Vaccinium macrocarpon*) or extracted from the fruit itself has been scientifically verified. Studies have shown that some compounds of Cranberry may also limit the prostate cancer through the inhibition of metalloproteinases¹¹. Ruel et al. (2009)¹² found that Cranberry juice consumption was associated with a decrease in plasma concentration of MMP-9 in obese. It has been shown that the cranberry extract has the ability to inhibit the adhesion to dental structures of *S. sobrinus*¹³, and reduces the *S. mutans* biofilm formation *in vitro*¹⁴, and the development of dental caries in vivo studies¹⁴. Furthermore, Bodet; Chandad; Grenier (2007)¹⁵

reported that low concentrations of cranberry extract are capable of inhibiting the secretion of MMP-3 and MMP-9 by the fibroblasts and macrophages after stimulation of agents responsible for periodontal problems. La, Howell; Grenier (2009)¹⁶ reported that cranberry was able to inhibit the production of MMPs in some inflamed periodontal and catalytic activity of MMP-1 and MMP-9 was also inhibited under these conditions.

In view of the above and considering that the components of Cranberry can inhibit metalloproteinases dentin exposed to erosive agents, thus minimizing the deleterious effects of erosion agents on dentin structure, this *in situ* study was designed to evaluate through two response variables (wear and surface hardness), the possible protective effect of a Cranberry based gel applied to the dentin subjected to the action of erosive agents.

MATERIALS AND METHODS

Experimental design

This *in situ* study was approved by the Research and Ethics Committee of the Bauru School of Dentistry, University of São Paulo (CAAE 04346612.3.00005417) and all the volunteers signed informed written consent. It was a crossover study performed in two phases of 5 days each, with a washout period of 7 days. Eleven volunteers wore acrylic palatal appliances each containing four dental enamel specimens randomly assigned into 2 rows. In the first phase specimens were subjected to erosive challenge (Coca-cola® over dentine without any previous treatment (G1; negative control 1) or erosive challenge over dentine previously treated with 0.05% Cranberry gel (G2; test group). In the second phase the specimens were subjected to erosive challenge over dentine previously treated with a gel without any active principle (G3; negative control 2) or erosive challenge over dentine

previously treated with 0.012% Clorexidine gel (G4; positive control). The phases were randomized. Half of the volunteers started the study in the first phase and the other half in the second phase. After the end of each phase, the volunteers crossed over to the other phase. The response variables were surface wear and change in surface hardness. Sample size calculation was based on a pilot study in order to provide an α -error of 5% and a power of 80%. Since the mean wear and change in hardness was quite different for the groups studied, ten volunteers were included, as in a previous report¹⁷.

Preparation of the enamel specimens

Dentin specimens (4x4x3 mm) were prepared from freshly extracted bovine incisors, which were stored and sterilized in 2% formaldehyde solution pH 7.0 for 30 days at room temperature. The surface of the specimens was ground flat with water-cooled carborundum discs (320, 600 and 1200 grades of Al₂O₃ papers; Buehler, Lake Bluff, IL, USA), and polished with felt paper wetted with diamond spray (1 μ m; Buehler), resulting in removal of about 100 μ m of the enamel, which was controlled with a micrometre. Surface Knoop hardness tests were performed at five sites in different regions of the specimens (15 g, 10 s; HMV-2000; Shimadzu Corporation, Tokyo, Japan) and 80 specimens with a mean surface hardness of 45 \pm 7 kp/mm² selected. The specimens were randomly divided into 4 groups. In order to maintain reference surfaces for lesion depth determination, two layers of nail varnish were applied on 2/3 of the surface of each specimen. The gels from different groups were applied over specimens one time before the first erosive challenge for 5 minutes. All gel formulations presented essentially the same composition¹⁸ except for the active vehicle of each group [0.012% Chlorhexidine,

Cranberry (Shaanxi M.R Natural Product Co.,Ltd)]

Palatal appliance preparation

Custom-made acrylic palatal appliances were made with four cavities (5x5x4 mm), two each in the left and right sides of the appliance. One specimen was randomly assigned to each of the four sites and fixed with wax. The position of each group in the appliance was randomly determined for each volunteer. In all groups the specimens were fixed so that the surface was at the same level as the appliance.

Intraoral phase

Ten healthy adult volunteers (all females, aged 19-30 years) residing in the same fluoridated area (0.70 mg F/L) took part in this study. Inclusion criteria were: normal salivary parameters, such as adequate stimulated and unstimulated flow rates (1.88 ± 1.00 mL/min and 0.60 ± 0.33 mL/min, respectively) and salivary pH (7.32 ± 0.30); freedom from erosive lesions or untreated carious cavities.

For each phase, the volunteers wore a new palatal appliance for five consecutive days. In the first 24 h of each intraoral phase, specimens were not subjected to acid challenges to allow the formation of a salivary pellicle⁶.

In both phases, the erosive challenges were performed ex vivo three times/day (7 a.m., noon and 6:00 p.m.). In each challenge, the appliance was immersed in a cup containing 150 mL of a freshly opened bottle of a cola soft drink for 5 minutes (Coke; Companhia Fluminense de Refrigerantes, Porto Real, Rio de Janeiro, Brazil). The drink was not degassed and had a pH of 2.6, contained 8.25% sucrose, 0.002% P and the blocks were immersed in it at room temperature. After that, the appliance was replaced into the mouth.

The volunteers received instructions to wear the appliances continuously, even at night, but to remove them during meals (3 times a day). In this period the appliance was stored in wet gauze. Oral hygiene was performed directly after meals when the appliance was not in the mouth. Throughout the experimental period the volunteers brushed their teeth with fluoride dentifrice (1,100 ppm F as NaF, pH 6.8; Crest, Procter and Gamble, Cincinnati, OH, USA).

Hardness

At the end of each experimental phase, the specimens were removed from the appliance and the nail varnish over the reference surfaces was carefully removed with acetone-soaked cotton wool. When the specimens were removed from the oral appliance, they were fixed on acrylic discs which were identified with random numbers. When the analyst measured the profilometry and the superficial hardness he thus did not know which group the specimens belonged to. The surface hardness of the enamel specimens was measured again using a hardness tester (Shimadzu HMV-2000, Shimadzu Corporation, Japan) with a Knoop diamond under a 15 g load for 10 s. Ten indentations were made on each specimen, five on the previously protected enamel surface (control), which was unaffected by the experimental period (SH₁) and five on the experimental areas (SH₂). The reduction in hardness (Percentage of Superficial Microhardness Loss; %SML) was calculated as $(SH_1 - SH_2) \times 100$.

Wear

The dentin samples were maintained wet until the analysis to avoid shrinkage of the organic layer. Immediately before the profilometric measurement, only the excess of water was carefully removed with filter paper. Prior to treatment, identification marks were made on the

sample surfaces using a scalpel, which allowed for accurate repositioning of the stylus. Subsequently, five baseline surface profiles were obtained from all the samples using a profilometer (MarSurf GD 25, Göttingen, Germany) at certain distances from the edge: 2.25, 2.0, 1.75, 1.5, and 1.25 μ m. The marks and two external thirds of the dentin surface were covered with nail varnish in order to allow reference surfaces for wear analysis. After five days of erosive cycling the nail varnish was removed and profilometric analysis was performed again at the same sites as the baseline measurements. The dentin loss was quantitatively determined using specific software (MarSurf XCR 20, Göttingen, Germany) by calculating the average depth of the eroded surface relative to the baseline surface profiles. Since the dentin samples could be precisely repositioned in the wells of the profilometer, it was possible to match the respective baseline and final profiles.

Statistical analysis

The assumptions of equality of variances and normal distribution of errors were checked for the tested response variables. Since the assumptions were satisfied, Repeated Measures Analysis of Variance followed by Fisher's test ($n=10$, $p<0.05$) were performed. The analyses were performed with STATISTICA 10.0 software (StatSoft Inc. Tulsa, OK, USA).

RESULTS

The % SML data showed no statistically significant differences between groups. On the other hand, there were differences in wear. It can be seen that there was no statistically significant difference in wear between the placebo and control groups (no gel). There was also no difference between the groups Chlorhexidine and Cranberry. However, the Chlorhexidine and Cranberry group

showed lower wear when compared to placebo and control groups.

DISCUSSION

This study evaluated *in situ* MMP inhibition capacity in dental erosion more specifically in dentin, using gels with two active compounds (Chlorhexidine and Cranberry). The erosion was performed extraorally with immersion in the acidic drink for 5 minutes to produce demineralization. It is likely that the low pH beverage has induced activation of MMP from dentin and saliva. It is generally considered that MMPs, although activated, cannot degrade the organic matrix of dentin in acid pH. Therefore immersions were conducted every three hours, to enhance the degradation of MMP matrix¹⁹, since the sound dentin undergoes an action by an acidic beverage, the pH falls, and occurs demineralization the inorganic component of dentine exposing collagen fibers and MMPs, and in this moment at which the pH is lower than the activation of MMPs occur, and it is when the pH is acid that occur the activation of MMPs, and when the pH returns to normal (pH 7.0) occurs the degradation of collagen fibrils by MMP⁴.

Chlorhexidine was chosen as the positive control for this study due

to its large number of studies that use this agent as inhibitors of MMPs in dentin for dental erosion purposes. Another active ingredient used in this work was the Cranberry, because this fruit being widely studied by medicine in the inhibition of MMPs, cancer cells, preventing the adhesion of host cells and inhibition of metabolic cells in obese^{11,21}.

In dentistry, the Cranberry has been studied in the prevention of tooth decay by inhibiting the production of organic acids and the formation of biofilms by cariogenic bacteria²¹. In periodontics, cranberry polyphenols act by reducing the inflammatory response, as well as the production and activity of proteolytic enzymes that contributes to the destruction of the extracellular matrix in periodontal disease²¹. The polyphenols also interfere Cranberry in various activities (including biofilm formation and adhesion) of *Porphyromonas gingivalis*, the main agent in chronic periodontitis²¹.

As the results found in this study, Magalhães et al. (2009)⁴ also found satisfactory results with respect to MMP inhibitors (Chlorhexidine, green tea), concluding that there was a significant difference between the groups, since the chlorhexidine and green tea statistically reduced tooth erosion compared to the control group.

This is also happened in the study of Kato et al. (2010)¹⁸ that evaluated the possible effect of EGCG (green tea extract) and chlorhexidine to prevent dental erosion in dentin and they concluded that the gels used for this work inhibit MMPs, by preventing tooth erosion in dentin.

Besides the Chlorhexidine there are other possible MMPs inhibitors being tested in dentistry, and many of these works have achieved a satisfactory result corroborating the findings of this study. As noted in the studies cited above, another MMP inhibitor that is being widely researched is green tea. In one of these studies Kato et al. (2009) evaluated the green tee protective effect on the dentin erosion and the authors concluded that green tea could prevent erosion in dentin. Thus, based on the results of this study, Cranberry gel may play an important role in the prevention of erosion in dentin, since it showed similar results when compared to the gold standard (chlorhexidine), without, however, provide much of the known side effects. So, these results suggest a significant efficacy of Cranberry gel in preventing wear of dentin subjected to dental erosion.

Table 1. Mean (\pm S.D.) of %SML and dentin loss (μ m) for the studied groups.

GROUP	%SML (Mean \pm SD)	WEAR (Mean \pm SD)
G1- No Gel	28.1 \pm 5.7 ^A	15.6 \pm 2.4 ^b
G2- Cranberry Gel	24.9 \pm 5.4 ^A	23.1 \pm 1.4 ^e
G3- Placebo Gel	25.7 \pm 9.1 ^A	15,1 \pm 2.3 ^b
G4- Chlorexidine Gel	29.8 \pm 8.6 ^A	15.2 \pm 2.5 ^b

*Different capital letters mean statistical differences between groups considering %SML and different lowercase letters mean statistical differences between groups considering wear (Repeated Measures ANOVA and Fisher's Test, p<0.05)

CONCLUSION

After analyzing the results, it can be concluded that:

There was a statistically significant efficiency of Cranberry and Chlorhexidine gels and in preventing wear of dentin subjected to dental erosion in relation to placebo and control groups. There were no significant differences %SML between studied groups.

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