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Protective effect of green tea on dentin erosion and abrasion

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Abstract

(1) Objective: This *in situ* study evaluated the protective effect of green tea on dentin erosion (ERO) and erosion-abrasion (ABR). (2) Design: Ten volunteers wore intraoral palatal appliances with bovine dentin samples subjected to ERO or ERO + toothbrushing abrasion performed immediately (ERO+I-ABR) or 30 min after erosion (ERO+30-min-ABR). During 2 experimental 5-day crossover phases, the volunteers rinsed with green tea or water (control, 1 min) between each erosive (5 min, cola drink) and abrasive challenge (30 s, toothbrushing), 4x/day. Dentin wear was measured by profilometry. (4) Results: The green tea reduced the dentin wear significantly for all conditions compared to control. ERO+I-ABR led to significantly higher wear than ERO, but it was not significantly different from ERO+30-min-ABR. The ERO+30-min-ABR provoked significant higher wear than ERO, only for the placebo treatment. (4) Conclusions: From the results it can be concluded that the green tea reduces the dentin wear under erosive/abrasive conditions.

Key words: Dentin, Green Tea, Tooth Erosion, Tooth abrasion

Introduction

Severe dental erosion is accompanied by dentin exposure, which might be associated with painful hypersensitivity and is accompanied by an increased risk for further dentin wear by different chemical and physical processes, such as erosion and abrasion (1).

In dentin, the erosive demineralisation results in the exposure of an outer layer of fully demineralised organic matrix followed by a partly demineralised zone until the sound inner dentin is reached (2). The degradation of the dentin matrix occurs after it has become accessible by the removal mineral, i.e., the dentin matrix cannot be degraded unless it is demineralised (3). The dentin demineralisation rate decreases when the amount of degradable collagen increases, whereby the demineralised matrix is attributed to hamper ionic diffusion into and out of the demineralising area (3, 4). However, the organic matrix can be degraded mechanically and chemically, which can contribute to an enhanced progression of dentin wear (5-7).

Among the proteases that can chemically degrade the organic matrix of dentin are matrix metalloproteinases (MMPs) present in dentin and saliva (8). MMPs are responsible for hydrolyzing the components of the extracellular matrix (ECM) during remodeling and degradation processes in the oral environment. MMPs related to the degradation of the collagen matrix of dentin are especially MMPs 2, 8, and 9 (8-10). MMPs get activated when the pH drops in the presence of acids from cariogenic challenges. The subsequent neutralization by salivary buffer systems enhances the degrading activity of the organic matrix (8). Besides, phosphorylated proteins released during the demineralisation of the organic matrix can interact with inhibited host MMPs within the lesion and reactivate them,

thus enhancing the degrading activity. The activation of MMPs seems to be important for the progression of dentin caries, since they have a crucial role in the collagen breakdown in caries lesions. Individuals with a high concentration of MMPs in saliva present an increased susceptibility to dental caries (11). Despite of a lack in studies investigating the role of MMPs in dental erosion, processes similar to those occurring for caries could be speculated to occur for erosive lesions. In this sense, the maintenance of the organic matrix on eroded dentin would be likely to postpone further erosion progression, which could be achieved by the use of MMP inhibitors. This strategy has been successfully employed, by using chlorhexidine as an MMP inhibitor, for the reduction of the degradation of the dentin hybrid layer both *in vivo (12)* and *in vitro* (13).

Due to the above-mentioned considerations, it might be interesting to find MMP inhibitors (14) that could play a role on the reduction of dental erosion. Green tea polyphenols, especially epigallocatechin-3-gallate (EGCG), were found to have distinct inhibitory activity against MMPs (11, 15-17). Thus, the aim of this *in situ* study was to test the protective effect of green tea on dentin erosion (ERO). Considering that the softened zone by erosive challenge seems to be more susceptible to mechanical forces, such as abrasion (18, 19), the protective effect of green tea on erosion associated to immediate abrasion (ERO+I-ABR) or abrasion 30 min after the erosive challenge (ERO+30-mim-ABR) was investigated as well.

Material and methods

Samples preparation

One hundred and twelve crown dentin samples (4 x 4 x 3 mm) were prepared from extracted bovine incisors. One sample was obtained from the labial surface of each crown. For preparation of dentin samples, enamel was completely removed until dentin was just exposed. The exposed dentin was ground flat with water-cooled carborundum discs and polished with felt paper wet with 1 µm diamond spray (Buehler), resulting in a removal of about 100 µm of the outermost layer. For allocation of the samples to the groups, the surface microhardness was determined by performing five indentations in different regions of the samples (Knoop diamond, 10 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). The overall range of microhardness was 55-75 KHN. Samples were allocated to groups by stratified randomization according to the mean surface microhardness. All groups presented similar mean microhardness (around 65 KHN).

In order to maintain reference surfaces for lesion depth determination by profilometry, two layers of nail varnish were applied on half of the surface of each sample. Individual acrylic palatal appliances with 6 palatal cavities were used for intraoral exposure of the samples. In three rows, each two samples were arranged on the left and right sides of the appliance.

Ethical aspects

This study was approved by the Institutional Review Board of Bauru Dental School, University of São Paulo, Brazil (Process 021/2007) and volunteers participated after signing an informed consent. Ten healthy adult volunteers (aged 20-30 years) residing in a fluoridated area (0.6-0.8 mg F/L) (20), who fulfilled the inclusion criteria described below, took part in this study. They presented normal salivary parameters, such as adequate stimulated and unstimulated salivary flows (> 1.0 and 0.5 mL/min, respectively) and

salivary pH (≥ 6.8). The subjects were free from erosive lesions, untreated carious cavities or periodontitis. The number of volunteers in the present study was defined according to previous studies (21-23).

Intraoral phase

This *in situ* study involved 10 volunteers and was performed in two crossover 5-day phases, with a washout period of 7 days. In the first 12 h of each intraoral phase, samples were not subjected to erosive and abrasive treatments to allow the formation of a salivary pellicle (24). On the following 5 days, erosive and abrasive challenges were made extraorally 4 times a day at predetermined times (8, 12, 16 and 20 h) after the principal meals.

For erosion of the dentin samples, the volunteers were instructed to remove the appliance and immerse it in a cup containing 150 mL of regular Coke (pH 2.6; Coca-cola Company Spal, Porto Real, RJ, Brazil) for 5 minutes at room temperature. During this period, the volunteers were instructed to prepare the green tea, according to manufacturers' instructions (Yamamotoyama, Midori Indústria de Chá Ltda, São Miguel Arcanjo-SP): infusion of 2 g of the herb (sachet) into 180 mL of boiled water (100^{o} C) for 1 minute. The tea was cooled off for 4 minutes in room temperature. The fluoride content of the tea was 0.87 mg fluoride/L and its pH, 7.0. The same procedure (including previous heating and cooling off) was performed with water from the public supply (0.6-0.8 mg fluoride/L), which was used as control. The temperature of the green tea and water immediately before the rinse was around 50°C.

After the 5 minutes of erosive challenge, the volunteers took one sip of the beverage. Thus, the appliances were re-inserted into the mouth and the volunteers rinsed

with 10 mL of green tea or water for 1 minute. Subsequently, the appliance was removed for abrasion treatment. While one row remained unbrushed (ERO), the other row was brushed *ex vivo* using a soft end-rounded electric toothbrush (Colgate Montions Multi-action, Brazil) with ~0.3 g of non-fluoridated dentifrice (Crest, Procter & Gamble, USA) for 30 s (166 oscillations/s) each sample (ERO+I-ABR). The appliances were replaced into the mouth and the volunteers rinsed with water (10 mL, 5 s). After 30 minutes of erosion, the abrasive treatment was repeated for the 3th row (ERO+30-min-ABR). The rows for the 3 experimental conditions were randomly allocated for each volunteer.

The volunteers received instructions to wear the appliances continuously for 24 h but to remove them during meals (4 times/day), when the appliance was stored in wet gauze. Seven days prior to the beginning and throughout the experimental phase, the volunteers brushed their teeth with a non-fluoridated dentifrice (Crest, Procter & Gamble, USA). They were also instructed to avoid licking of the samples with the tongue to avoid abrasion (25). The volunteers received oral and written information to refrain from using any fluoridated or antibacterial product.

Wear analysis

After the *in situ* phase, the samples were removed from the appliances and kept moistened (gauze soaked in water) up to the wear analysis in order to avoid shrinkage of the dentin organic material. The nail varnish over the reference surfaces was carefully removed (19). The dentin wear was determined in relation to the reference surfaces by contact profilometry (Hommel Tester T 1000, Hommelwerke, VS, Schwenningen, Germany). Five readings were performed on each sample by scanning from the reference to the exposed surface. The mean values of five readings for each group were averaged.

Statistical Analysis

The assumptions of equality of variances and normal distribution of errors were checked for all the variables tested, using the Bartlett and Kolmogorov-Smirnov tests, respectively. Since the assumption were satisfied, two-way repeated measures ANOVA and Bonferroni *post hoc* test were used. The factors evaluated were treatment (green tea or water) as the dependent variable and condition (ERO, ERO+I-ABR and ERO+30-min-ABR) as the independent variable. The significance level was set at 5%. The software GraphPad Prism 4 version 4.0 for Windows, Graph Pad Software (San Diego, CA, USA) was used.

Results

There was a significant difference among the conditions ($F=4.50$, $p = 0.021$) and between the treatments ($F=65.45$, $p < 0.0001$). Table 1 shows that the green tea significantly reduced the dentin wear for all conditions ($p<0.01$). For both treatments, the wear was significantly higher when abrasion was performed immediately after erosion (ERO+I-ABR) when compared to erosion alone (ERO). Regarding delayed abrasion (ERO+30-min-ABR), when rinsing was performed with water (control), a significantly higher wear was observed when compared to erosion alone (ERO). This did not happen for the green tea rinse which led to wear values that did not significantly differ from ERO. No significant differences in wear were observed when the conditions ERO+I-ABR and ERO+30-min-ABR were compared, for both treatments (Table 1).

Discussion

The present study tested the effect of green tea on dentin wear by erosive/abrasive processes. The response variable chosen to assess this effect was contact profilometry, which is widely used for this purpose (19, 26-29). In order that this methodology can be used, it is essential to keep the specimens moistened before and during analysis, as it was done in the present study, in order to avoid shrinkage of collagen fibrils which could interfere in the results. The green tea significantly reduced the dentin wear under erosive/abrasive conditions. Despite using a mechanical stylus, which is expected to cave into the organic material to some extent and give higher values than obtained with an optical device (7), the amount of wear (0.98 μm loss after 100 min of erosive challenge) obtained in the present study was low when compared to previous studies from our group (3.6 μm loss after 28 min of erosive challenge) (19). This can be explained by the different sensitivities of the equipments and the software used in both studies.

The protective effect of green tea could not be attributed to its fluoride content, since it was quite similar to that present in the negative control (water from the public supply). Additionally, it also could not be attributed to the temperature of the rinse, since the water rinse had the same temperature. One possible mechanism of action of green on the reduction of dentin erosion could be the inhibition of MMPs. If it is true, the main responsible for this effect may be the polyphenols. Green tea polyphenols, especially epigallocatechin-3-gallate (EGCG), were found to have potent and distinct inhibitory activity against MMPs in cell culture tests (15, 16). EGCG seems to exhibit a hydrogen bonding and hydrophobic interaction with collagenases, which is responsible for the change in the secondary structure of collagenases and consequently for their inhibition (31). The EGCG concentration in the green tea used in the present study was 0.185 mg/mL, as evaluated using high-performance liquid chromatography (data not shown),

which is quite above the reported IC_{50} values for the inhibition of MMP-2 and MMP-9, which were 10 and 0.6 µg/mL of EGCG, respectively (16).

The *in situ* model used in this study allowed the formation of an acquired salivary pellicle which might play an important role during the erosive challenge (24). Considering the possibility that green tea would inhibit MMPs activity and in turn allow the maintenance of an organic layer on the eroded dentin, this model would also allow not only the MMPs from dentin, but also from saliva, to influence the dentin wear. It is known that the saliva-derived MMPs could be involved in the destruction of the organic matrix (8, 30) and that the MMP-8 present in saliva may negatively influence the remineralisation of demineralised dentin (31).

Erosion was produced by a cola drink, as it is one of the most widely consumed soft drinks and exhibits erosive potential (21, 22). Erosion was performed by extraoral immersion in cola for 5 min, in order to produce the demineralisation. It is also probable that the low pH of the drink has induced the activation of dentin-derived MMPs and saliva-derived MMPs, when the volunteers drank one sip of the beverage (8). However, it is generally regarded that MMPs, although activated, cannot degrade the organic matrix of dentin at acidic pH (8). In this protocol, the interval between each erosive challenge (>2h), could allow for remineralisation and also pH neutralisation, which is essential for enhancing the degrading activity of the organic matrix by MMPs. However, it must be acknowledged that the protocol employed in the present study does not allow the conclusion that the effect of green tea on reducing the wear of dentin specimens was due to its inhibitory effects on MMPs activity, since we did not test this directly. Further studies focusing on the determination of the activity of MMPs in the organic layer overlying the eroded dentin after an erosive challenge could be instructive on this matter. The

verification of the organic material on dentin surface by SEM could also bring additional useful information.

Tooth wear is a multifactorial condition caused by chemical (erosion) and mechanical (abrasion and attrition) processes, since the softened zone by erosive challenge is more susceptible to mechanical forces, such as abrasion (18, 19). Thus, studies regarding tooth wear have to consider both chemical and mechanical challenges to simulate the clinical situation. This aspect is especially important in eroded dentin, where the exposed organic matrix acts as a protective layer against further demineralisation, and excessive toothbrushing could impair this matrix. However, a recent report suggested that the demineralised organic layer developed after erosive challenges was unaffected by brushing (7). To simulate abrasive conditions which might occur during oral hygiene treatment, brushing abrasion of each specimen was performed for 30 s each cycle.

Regarding the abrasive wear of eroded dentin, the present results confirm previous studies showing that abrasion subsequently after an erosive attack can increase wear of acid-softened tooth surfaces (32, 33). However, 30 min delay of abrasion was not able to reduce dentin wear compared to brushing immediately after erosion, which was also shown in previous studies (18, 34). Thus, in the clinical situation, the delay of 30 min in toothbrushing after erosion might be ineffective on reduction of dentin wear.

From the results of the present study, it can be concluded that the green tea reduces the dentin wear under erosive/abrasive conditions, but additional studies are required to confirm its mechanism of action on this process.

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Table 1. Wear (µm) of dentin samples subjected to erosion or erosion + abrasion (immediate or after 30 min) treated with green tea or water. Mean ± SD (95% CI)

	Conditions		
Treatment	**Erosion**	**Erosion + immediate abrasion**	**Erosion + 30-min-abrasion**
Water[A] (Control)	0.98±0.13[a] (0.89-1.07)	1.23±0.35[b] (0.98-1.48)	1.22±0.23[b] (1.05-1.38)
Green tea[B]	0.59±0.18[a] (0.46-0.72)	0.90±0.32[b] (0.67-1.13)	0.74±0.23[ab] (0.57-0.91)

Distinct upper case letters indicate significant differences between the treatments. For each row, distinct lower case letters indicate significant differences among the conditions (2-way repeated-measures ANOVA, $p<0.05$).