The present study evaluated the effect of NaF and CPP-ACP/NaF varnishes to reduce erosion produced by soft drink (SD) combined or not with pediatric liquid medicine. Enamel specimens were pre-treated with fluoride varnish, according to the following groups: NaF varnish (Duraphat®) or CPP-ACP/NaF varnish (MI varnishTM). Two types of erosive cycles were made: by soft drink erosion (SDE) or by pediatric liquid medicine plus soft drink erosion (PLM/SDE). Bovine enamel specimens were randomly assigned in six groups (n=10): G1=NaF + SDE; G2=CPP-ACP/NaF + SDE; G3=Distilled and deionized (DD) water + SDE; G4=NaF + PLM/SDE; G5=CPP-ACP/NaF + PLM/SDE and G6=DD water + PLM/SDE. Before treatments, the sample surface was divided in two areas (unexposed area-UA and exposed area-EA). The specimens were evaluated by 3D non-contact profilometry technique to determinate tooth structure loss (TSL) and surface roughness (Sa). Scanning electron microscopy (SEM) analysis was also performed. After SDE, G2 presented the lowest TSL values compared to G3 (p=0.008). G1 and G2 did not differ between them (p=0.203) and no groups differed among them despite Sa. Regarding TSL and Sa, G4 and G5 differed from G6 (p=0.0001), but not between them (p=1.00). Examining 3D and SEM images, the greatest differences between UA and EA were observed for G3 and G6. CPP-ACP/NaF varnish seems to be a promising treatment to reduce enamel loss from the erosion produced by a soft drink. Both varnishes also showed capacity to reduce TSL and Sa after erosion by soft drink combined to pediatric liquid medicine.

Introduction

Dental erosion is the dissolution of teeth minerals by acids with no bacteria involvement. The chemical wear results in a roughened structure and the loss of tissue that may appear like a visible defect on dental surface (1,2). Dental erosion has a high prevalence, mainly among children and adolescents (3) and it has extrinsic or intrinsic causes (4).

The extrinsic causes include consumption of acidic foods or drinks and use of acidic medicines or acidic hygiene products (5). Liquid medicines are widely used for children because the facility of ingestion (5,6). However, some of inactive acidic components have low pH (5,7) and because of the high frequency of medication intake, bedtime consumption, high viscosity and reduction of the salivary flow, it may also be associated with alterations on surface morphology of dental enamel (5).

Some preventive actions have been suggested to avoid the start of the erosive process, like using professional topical fluorides (8,9). Furthermore, the addition of new remineralizing compounds to fluoride varnishes may be an alternative to obtain better effects against erosive wear.

Casein Phosphopeptide-Amorphous Calcium Phosphate Nanocomplexes (CPP-ACP) is the Recaldent™ technology based on stabilization of amorphous calcium phosphate (ACP) by casein phosphopeptides (CPP). It has been reported that the CPP-ACP nanocomplexes provide high concentration of calcium and phosphate ions. CPP-ACP compound can interact with fluoride ions to produce an ACPF phase and provide better benefits to teeth (10,11).

To the best of the authors' knowledge, the influence of CPP-ACP/NaF varnish on erosion prevention has not yet been investigated. Therefore, the purpose of this in vitro study was to evaluate the effect of CPP-ACP/NaF varnish compared with NaF varnish to provide protection against the erosion produced by soft drink combined or not with pediatric liquid medicine.

Material and Methods

Specimen Preparation

Enamel specimens (4x4x2 mm) were prepared from the labial surfaces of bovine incisors crowns. The specimens were cut using an ISOMET low-speed saw (Buehler Ltd, Lake Bluff, IL, USA) with 2 diamond discs (Extec Corp, Enfield, CT, USA) with a 4-mm spacer. The specimen’s surface was polished using water-cooled silicon carbide paper 600 and 1200 (Extec Corp.). After each polishing
phase, the specimens were cleaned in an ultrasound device with distilled and deionized (DD) water (Milli-Q®, Merck Millipore Corporation, Darmstadt, Germany) for 5 min. The specimens were checked regarding the presence of white spots and cracks using a microscope (40×).

Baseline surface roughness (Sa) of the enamel surface was measured (Sa=1.82±0.18 μm), using a 3D noncontact chromatic confocal optical profilometry (Nanovea PS50 Optical, NANOVEA Inc., Irvine, CA, USA). An area of 1×1 mm was delimited in the center of each sample. Three scans for each area (200×200 μm) were acquired and the average was used to determine the Sa.

Prior to the experiment, a nail varnish was applied in the right half of the specimen’s surface to maintain a sound reference surface (unexposed area, self-control per specimen), the other half of the surface (left side) was not covered, representing the exposed area. The specimens were maintained in 100% humidity until the beginning of the experiment.

**Treatment and Erosive Cycling**

The fluoride varnishes utilized in this study were: (1) NaF varnish (5% NaF, Duraphat®, Colgate Oral Pharmaceuticals, New York, NY, USA) and (2) CPP-ACP/NaF varnish (2% CPP-ACP and 5% NaF, MI Varnish™, GC America, Alsip, IL, USA).

Sixty enamel specimens were randomly allocated to 6 groups (n=10) according to treatment and type of erosion challenge: G1= NaF varnish + soft drink erosion; G2= CPP-ACP/NaF varnish + soft drink erosion; G3= DD water + soft drink erosion (negative control for soft drink erosion); G4= NaF varnish + pediatric liquid medicines/soft drink erosion; G5= CPP-ACP/NaF varnish + pediatric liquid medicine/soft drink erosion; G6= DD water + pediatric liquid medicine/soft drink erosion (negative control for pediatric liquid medicines + soft drink erosion).

The sample size of 10 specimens was based on a 0.8
power to detect a significant difference of 50% in tooth structure loss (TSL) in each varnish group compared to negative control, using a one-sided test considering a 5% error level and 20% b-error level (BioEstat 5.3, Instituto de Desenvolvimento Sustentável Mamirauá, Tefé, AM, Brazil).

In groups G1, G2 and G3 soft drink erosion (SDE) were performed with Coca-Cola® (Coca-Cola® Company, Porto Real, RJ, Brazil) and in G4, G5 and G6, with pediatric liquid medicine plus soft drink erosion (PLM/SDE) were performed immersing the specimens in Claritin® (Schering-Plough, Kenilworth, NJ, USA) and in Coca-Cola® (Fig. 1).

The varnishes in G1, G2, G4 and G5 groups were applied once at the beginning of the experiment in a thin layer on specimen surface, using a microbrush. After 6 h of immersion in artificial saliva (1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl, 0.05 mg F/mL in 0.1 mol/L Tris buffer, pH 7.0, 25 mL/sample) (12,13), the layer of varnish was removed using a scalpel blade and acetone with water (1:1) (13-15), the total removal was checked microscopically ([40].

In order to simulate the clinical situation, all samples were subjected to erosive cycles for 4 days. All groups were submitted to 6 SDE per day for 10 min each (16), using freshly opened bottles of Coca-Cola® pH 2.58, 15 mL/specimen. Furthermore, the samples of G4, G5 and G6 were also immersed 2x/day (before the first and last soft drink immersion) for 5 min in a pediatric liquid medicine (Claritin®) (17), pH 2.12, 15 mL/specimen. Between each SDE, the samples of all groups were immersed in artificial saliva. After each erosive period, samples were rinsed in DD water. The experiment was carried out at 37 ºC (Fig. 1).

**Profilometric Analysis**

After 4 days of experiment, the layer of acid-resistant nail varnish was removed using acetone with water (1:1) (13-15), allowing the comparison between the unexposed and exposed areas.

A chromatic confocal sensor with a white light axial of 3D non-contact optical profilometer scanned an area of 1x1 mm on each specimen (velocity of 2 µm/s) and generated one image for each sample. The images were analyzed by Nanovea Professional 3D software (Nanovea PS50 Optical, NANOVEA Inc.) to determine tooth structure loss (TSL) and surface roughness (Sa), according to previous studies (13,17).

Tooth structure loss (µm) was evaluated at the center of each specimen; three 1-mm-long linear measurements were made involving the unexposed and exposed area to calculate the difference in height between the unexposed and exposed enamel surface in each specimen. All measurements were done in triplicate, and the average was used to represent the final result of the specimen’s surface profile. To determine Sa, three scans (200 µm×200 µm) were acquired in each area (unexposed and exposed areas) on the enamel specimen. The average of these three measurements in each area was used to determine Sa1 (surface roughness in unexposed area) and Sa2 (surface roughness in the exposed area), the Sa value for all groups was calculated as the difference between Sa1 and Sa2 by using the following formula: Sa = Sa1 – Sa2.

**3D Profilometry Images and Scanning Electron Microscopy (SEM)**

One 3D topographical image was chosen to represent the results of each group. Two SEM micrographs of each group were also obtained.

3D topographical images were constructed from scanned area (1x1 mm) by Nanovea Professional 3D software, all images were standardized in height parameter. SEM analysis was performed in scanning electron microscope (6460LV, JEOL, Akishima, Tokyo, Japan). Three enamel specimens of each group were randomly selected, covered with a 30-µm gold layer and fixed on stubs with double-faced carbon tape. The topography of enamel specimens was analyzed by backscattered electrons at 20 kV in low vacuum mode (45 Pa). The SEM micrographs at x500 magnification were used to observe the interface between areas (unexposed and exposed) and those at x2000 magnification were used to have a more detailed view of the surface alterations. The schematic design of the experimental protocol is shown in Figure 1.

**Statistical Analysis**

The normal distribution of data was checked for all of the tested variables, using the Shapiro Wilks test. Differences in TSL and Sa among treatments were tested using one-way ANOVA followed by Tukey test. SPSS software version 22.0 (IBM, New York, NY, USA) was used for statistical analysis. The significance level was set at 5%.

**Results**

Table 1 summarizes the 3D non-contact profilometry results for TSL and Sa values after SDE challenge. The specimens of G1 and G2 did not differ between each other (p=0.203) and only G2 differed statistically from G3 (p=0.008) showing lower average value for TSL (26.68±3.93 µm). About alterations in surface roughness, G1, G2 and G3 did not differ among them (p=1.00).

Evaluating the PLM/SDE challenge results, regarding TSL and Sa, both G4 and G5 did not differ between them (p=1.00), but differed statistically from G6 (p=0.0001). These results are in Table 2.

The differences in the magnitude of treatment surface effect can be seen on the 3D topographical (Fig. 2) and
SEM images (Figs. 3 and 4). Figure 2 shows the topographic characteristics of the surface enamel at the end of the experiment. The greater the areas in blue, the greater the tooth structure loss. In contrast, the greater the areas in red in the images, the smaller the tooth structure loss.

All groups had tooth structure loss and a significant increase in surface roughness. However, G3 and G6 groups showed a greater difference between unexposed and exposed areas. This issue became evident by the color. Color variation was less evident in the other groups and when G1 and G2 were compared with G3 as well as when G4 and G5 were compared with G6.

The same features were observed in the images obtained by SEM analysis (Figs. 3 and 4), in which G3 and G6 presented the worst alterations in enamel topography with great depressions/craters and exposure of enamel prisms.

**Discussion**

The irreversible loss of tooth tissue by exogenous or endogenous acids is characteristic of dental erosion (1,2). This process results in a progressive softening of the superficial and near-surface layer of enamel (4,8).

Some pediatric liquid medicines have certain acidic components that offer chemical stability, tonicity or improve their flavor (7), which can contribute to increase their erosive potential. Many of these pediatric medicines are usually used in treatments for long periods and are consumed daily (18). In addition to this fact, many children also use daily carbonated soft drinks that have low endogenous pH and may also contribute to dental erosion. Therefore, it is important to evaluate products that can reduce the erosion caused by soft drinks and by soft drinks consumed together with pediatric medicines to reproduce as close as possible the real situation.

In a previous study (17), our research group evaluated the influence of pediatric liquid oral medicines on enamel topography, and concluded that the majority of the studied pediatric medicines have low pH, mainly at room temperature; and Claritin® demonstrated in vitro the capacity to create significant gaps between the unexposed and exposed areas.

**Table 1.** Mean of tooth structure loss (TSL) and surface roughness (Sa) (±SD) of enamel specimen groups after soft drink erosion challenge

<table>
<thead>
<tr>
<th>Groups</th>
<th>3D non-contact profilometry results</th>
<th>TSL</th>
<th>Sa</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (NaF varnish)</td>
<td>30.01 ± 4.87 ± a,b</td>
<td>0.73 ± 0.62 a</td>
<td></td>
</tr>
<tr>
<td>G2 (CPP-ACP varnish)</td>
<td>26.68 ± 3.93 ± a</td>
<td>0.64 ± 0.43 a</td>
<td></td>
</tr>
<tr>
<td>G3 (DD water)</td>
<td>32.85 ± 3.10 ± b</td>
<td>0.86 ± 0.53 a</td>
<td></td>
</tr>
</tbody>
</table>

Means followed by distinct letters are statistically different (p<0.05).

**Table 2.** Mean of tooth structure loss (TSL) and surface roughness (Sa) (±SD) of enamel specimen groups after pediatric liquid medicine plus soft drink erosion challenge

<table>
<thead>
<tr>
<th>Groups</th>
<th>3D non-contact profilometry results</th>
<th>TSL</th>
<th>Sa</th>
</tr>
</thead>
<tbody>
<tr>
<td>G4 (NaF varnish)</td>
<td>31.12 ± 4.73 ± a</td>
<td>0.58 ± 0.49 a</td>
<td></td>
</tr>
<tr>
<td>G5 (CPP-ACP varnish)</td>
<td>27.69 ± 7.26 ± a</td>
<td>0.57 ± 0.24 a</td>
<td></td>
</tr>
<tr>
<td>G6 (DD water)</td>
<td>44.17 ± 8.55 ± b</td>
<td>1.69 ± 0.32 b</td>
<td></td>
</tr>
</tbody>
</table>

Means followed by distinct letters are statistically different (p<0.05).

Figure 2. 3D profilometry images of enamel surface samples after treatment and erosion challenges for each group. A-C images = soft drink erosion challenge, D-F images = pediatric liquid medicine + soft drink erosion challenge. (A) and (D) G1 and G4= NaF varnish, (B) and (E) G2 and G5= CPP-ACP/NaF varnish, (C) and (F) G3 and G6= DD water (negative control). 1= exposed area (after erosion), 2= the unexposed area (sound enamel).
and exposed enamel surface besides the increased surface roughness. The present study provides additional data to our previous study (17), which evaluated the effect of NaF and CPP-ACP/NaF varnishes to inhibit tooth structure loss and alterations in surface roughness after consumption of soft drinks and after pediatric liquid medicine plus soft drink erosion. Based on those previous results (17), Claritin® was chosen to simulate the erosive effects of acid drug

Figure 3. SEM micrographs of enamel surface samples after treatment and erosion challenges at 500. A-C images = soft drink erosion challenge, D-F images= pediatric liquid medicine + soft drink erosion challenge. (A) and (D) G1 and G4= NaF varnish, (B) and (E) G2 and G5= CPP-ACP/NaF varnish, (C) and (F) G3 and G6= DD water (negative control). 1= exposed area (after erosion), 2= unexposed area (sound enamel).

Figure 4. SEM micrographs of enamel surface samples after treatment and erosion challenges at 2000. A-C images = soft drink erosion challenge. D-F images= pediatric liquid medicine + soft drink erosion challenge. (A) and (D) G1 and G4= NaF varnish, (B) and (E) G2 and G5= CPP-ACP/NaF varnish, (C) and (F) G3 and G6= DD water (negative control). Arrow 1= exposure of enamel prisms, arrow 2= small depressions on enamel surface, arrow 3= larger depressions/craters.
caused by pediatric liquid medicine in erosive challenge of this study. Other in vitro studies have also demonstrated that Claritin® may reduce enamel hardness (18-20) and increase surface roughness (17).

In the present study, the varnishes were applied once and removed after 6 h of application to simulate the clinical condition, since the emphasis was allowing the chemical effect of the varnish components, rather than the mechanical protection. Because of their common clinical use, high fluoride amount and slow components release, varnishes could be an ideal product to be used as a preventive product against dental erosion (21).

As previously proposed by Sales-Peres et al. (16), a severe erosive challenge (6 SDE/day) was undertaken in the present study to verify the potential of the treatments under an extreme condition. This protocol was able to provide fast enamel demineralization as well as to simulate a high erosive risk situation, as seen in the present study. Tests using models that mimic oral conditions are required in the studies, so the sample was immersed in a pediatric liquid medicine for 5 min according to our previous study (17), to simulate the period in which the syrup remains in the oral cavity until its complete ingestion and dilution by the saliva.

The outcomes present study revealed that application of topical CPP-ACP/NaF varnish was effective to decrease tooth structure loss after soft drink erosion. Furthermore, both varnishes reduced tooth structure loss and enamel roughness after pediatric liquid medicine plus soft drink erosion. This is an important result because many children use for long periods antihistaminic medicines such as Claritin® and use of these varnishes could be a good alternative to protect against tooth structure loss and roughness alteration in enamel.

The literature explains the positive results of fluoride varnishes by the formation of a protective barrier of CaF2-like layer on dental tissue that inhibits the contact of acid with enamel and also help the de-remineralization process (22,23).

The NaF varnish demonstrated capacity to inhibit TSL only after PLM/SDE challenge. It may be speculated that this result may be combined to the erosive protocol. After a great erosive challenge, the protective effect of NaF varnish became more evident.

CPP-ACP/NaF varnish reduced tooth structure loss and enamel roughness alterations after both erosive challenges, probably because calcium and phosphate ions available in CPP-ACP/NaF varnish penetrate in enamel causing ion oversaturation (24). In addition to CaF2 action, the ACP group from CPP-ACP bonds with F- from NaF to produce the ACFP phase, which is unstable and rapidly transforms into fluorhydroxyapatite (25).

Also, the casein in this varnish could modify certain enamel mechanical properties, making it less susceptible to mineral loss and reducing the erosive process (25). Other previous studies (26,27) also observed that CPP-ACP/NaF toothpaste showed the best potential to control dental erosion demonstrating the synergy between CPP-ACP and fluoride.

In the 3D topographical and SEM images, the groups treated with CPP-ACP/NaF varnish had the least enamel topography alterations, probably due high bioavailability of calcium contributes to protect enamel surface, making it less irregular even under erosive challenges.

Despite its advantages, such as providing investigation in a controlled environment with a single-variable, this in vitro protocol has limitations, particularly related to its inability to adequately simulate the complex biological dental erosion processes, due the difficulty for matching the solid/solution ratios that occur in vivo. Nevertheless, this study is the first to evaluate the changes in surface enamel topography relative to surface roughness profile and tooth structure loss after erosive challenge from acidic pediatric liquid medicines.

Topical application of CPP-ACP/NaF varnish was effective in improving protection against tooth structure loss after soft drink erosive challenge, and both varnishes were able to reduce tooth structure loss and enamel roughness alteration after pediatric liquid medicine plus soft drink erosive challenge. These findings provided a basis for future in situ studies and clinical trials that can evaluate the effect of these varnishes in clinical erosive tooth wear, especially in cases of patients who use acidic pediatric liquid medicines.

**Resumo**

O presente estudo avaliou o efeito dos vernizes de NaF e CPP-ACP/NaF na redução da erosão promovida por refrigerante e associada a um medicamento líquido pediátrico. Os espécimes de esmalte foram prontamente submetidos a um esmalte fluoretado de acordo com o grupo de alocação: verniz NaF (Duraphat®) ou verniz CPP-ACP/NaF (verniz MITM). Dois tipos distintos de desafio erosivo foram realizados: erosão com refrigerante (ER) ou erosão com medicamento líquido pediátrico e refrigerante (MLP/ER). Espécimes de esmalte bovino foram aleatorizados em seis grupos (n=10): G1 = NaF + ER; G2 = CPP-ACP/NaF + ER; G3 = Água destilada e deionizada (DD) + ER; G4 = NaF + MLP/ER; G5 = CPP-ACP/NaF + MLP/ER; G6 = DD água + MLP/ER. Antes dos tratamentos, a superfície das amostras foi dividida em duas áreas (não exposta-Ne e área exposta-AE). Os espécimes foram avaliados pela técnica de perfilometria 3D de não-contato para determinar a perda de estrutura dentária (PED) e a rugosidade superficial (RS). A microscopia eletrônica de varredura (MEV) também foi utilizada. Após ER, G2 apresentou os menores valores de PED comparado ao G3 (p=0,008). G1 e G2 não diferiram entre si (p=0,203) e não houve diferença entre os grupos no que diz respeito a RS. Os resultados de PED e RS para a MLP/ER mostraram que G4 e G5 diferiram de G6 (p=0,001), mas não diferiram entre si (p=1,00). Examinando as imagens 3D da perfilometria e de MEV, as maiores diferenças entre UA e EA foram observadas para G3 e G6. O verniz CPP-ACP/NaF parece ser um tratamento promissor para reduzir a perda de esmalte por erosão produzida por refrigerante.
e ambos os vernizes mostraram capacidade em reduzir a PED e RS após erosão com medicamento líquido pediátrico associado a refrigerante.

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