Effect of CPP-ACP and CPP-ACPF Pastes on the Surface Hardness of Initial Dental Erosion Lesions: An In Situ Study

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To analyze the effect of intra-oral application of CPP-ACP and CPP-ACPF pastes on the surface hardness of initial dental erosion lesions. Material and Method: Bovine enamel specimens were randomly assigned into 6 volunteers in 3 treatment groups: GI: CPP-ACP paste, GII: CPP-ACPF paste and GIII (Control): Placebo paste without CPP-ACP and without fluoride. Enamel specimens were selected by surface hardness (SHi), in vitro eroded by immersion in hydrochloric acid for 30 seconds (SHdes) and randomized between treatment groups and volunteers, who used the palatal intraoral device for 2 hours, applied the treatment on the specimens and used the palatal intraoral device for an additional 3 hours in 3 crossed phases, interspersed with a 7-day washout period. Subsequently, the surface hardness (SHre) was measured to estimate the re-hardening potential of the softened enamel promoted by treatments. Data were analyzed using the t-test and one-way ANOVA, adopting 5% significance level. Results: Mean final and post-erosion hardness values were statistically significant for pastes tested (p<0.05), which presented re-hardening effect of the softened enamel, but with no difference between them and placebo (p>0.05). Conclusion: CPP-ACP and CPP-ACPF pastes did not demonstrate higher efficacy in re-hardening the eroded enamel compared to placebo paste.

Keywords: Tooth Erosion; Caseins; Toothpastes.

Dental erosion is characterized as a progressive and non-reversible loss of tooth structure caused by chemical processes that do not involve bacterial action1. It is a condition that involves the interaction of chemical factors, biological factors and behavioral factors2-6.

When in contact with the dental surface, acids cause the dissolution of the inorganic hard tissue7-9, causing decreased mechanical resistance and reduced hardness of the surface layer10. In this way, dental erosion can be divided into two phases: Phase 1: Erosion (chemical process), with partial demineralization of the enamel or dentin, causing surface softening; Phase 2: Erosive tooth wear, which characterizes the advanced phase, where there is loss of the previously softened surface through the combined effect of erosion and mechanical wear of the tooth surface11-13.

The use of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is among the strategies to control tooth erosion, a milk protein substance consisting of casein...
phosphopeptides (CPP) aggregated with calcium phosphate to form agglomerates of amorphous calcium phosphate (ACP)\textsuperscript{14,15}. CPP-ACP acts as a supplemental source of calcium and phosphate bioavailable in the oral environment, being proposed both for inhibiting loss of minerals\textsuperscript{1} and for intensifying their deposition on demineralized lesions of the dental enamel, such as erosive lesions\textsuperscript{16}.

Thus, pastes containing CPP-ACP can be considered as potential alternatives for the mineral deposition process \textsuperscript{17}, being an additional reservoir of calcium and phosphate ions in the oral environment\textsuperscript{16}. Furthermore, the addition of fluoride to CPP-ACP, forming CPP-ACPF, has been studied due to its potential to enhance the protective effect promoted by CPP-ACP in the control of dental erosion\textsuperscript{19,20}. However, the benefit of this association, to the best of our knowledge, still generates discussions\textsuperscript{1}, because literature is not consensual regarding its superiority with respect to dental erosion.

Considering the importance of the subject and the scarce literature currently available on the efficacy of CPP-ACP pastes on mineral precipitation of initial erosive lesions, and knowing that \textit{in situ} studies better mimic the clinical reality to obtain scientific information\textsuperscript{21}, the aim of this study was to analyze the \textit{in situ} effect of a single intrabuccal application of CPP-ACP and CPP-ACPF pastes on the surface hardness of initial erosion lesions in bovine enamel. The null hypothesis tested was that there is no difference in recovery of the enamel hardness by the use of CPP-ACP pastes and placebo.

**MATERIALS AND METHODS**

**Experimental Design**

A crossover, double blind, \textit{in situ} experimental study was carried out. The factor under study was mineral precipitation in dental erosion lesions according to groups: GI = 10\% CPP-ACP paste (MI Paste\textsuperscript{TM}, GC America Inc, USA); GII = 10\% CPP-ACP paste + 900ppm sodium fluoride NaF (MI Paste Plus\textsuperscript{TM}, GC America Inc., USA); and GIII = Paste without CPP-ACP and without sodium fluoride (Dilecta Farmácia de Manipulação & Homeopatia, João Pessoa, Paraíba, Brazil).

Bovine tooth enamel specimens (n = 36) were randomly divided among volunteers (n = 6), who used a palatal device for intraoral paste application. Each group was conducted in a 5-hour phase and the response variable used was surface hardness (hardness recovery percentage).

**Preparation of Specimens and Surface Hardness Evaluation**

Seventy-one bovine enamel specimens (4x4x3mm) were kept in 0.1\% thymol solution, pH 7.0\textsuperscript{22}, planned and polished with water sands (granulations 600, 1200, 1500, 2000) (3M Company, Minnesota, USA).

The initial surface hardness (SH\textsubscript{I}) was determined from the mean values of 5 indentations per specimen in the central region of the specimen and corresponding to the mean values of 5 indentations performed in the central region of the specimen at 100 times magnification (Vickers tip, 100g, 15s, Michohardness Tester FM-700, Future-Tech Corp., Fujisaki, Kawasaki-ku, Japan)\textsuperscript{22}. Thirty-six specimens with hardness from 266.94 KgF/mm\textsuperscript{2} to 375.42 KgF/mm\textsuperscript{2} were selected, which were sterilized by exposure to ethylene oxide gas\textsuperscript{22}.

Erosion lesion was performed \textit{in vitro} by immersing the enamel specimens in 150 ml of 0.01M hydrochloric acid (Vetec Química Fina Ltda., Rio de Janeiro, Brazil) at pH 2.3 with constant stirring for 30 seconds. Then, each specimen was washed with deionized water in order to stop the demineralization process\textsuperscript{23}.

For the evaluation of surface hardness after erosion (SH\textsubscript{des}), the same parameters used to determine the initial surface hardness were adopted. The average hardness (initial hardness of the healthy enamel and after erosion) was used to calculate the hardness loss percentage according to the following formula: \[(\text{initial hardness} – \text{post-erosion hardness}) / \text{initial hardness}] \times 100\textsuperscript{24}, whose values were used for the stratified random distribution of enamel specimens between volunteers and study groups, so that all the study groups, specimens with lower and higher hardness were allocated. The location of specimens on the right or left side of the device was also randomly determined for each volunteer and each phase of the study\textsuperscript{24}.

**Selection of Volunteers and \textit{in-situ} Experiment**

Six volunteers (aged 18-25 years), who met the inclusion criteria (stimulated salivary flow e”1 ml / min, adequate oral health, absence of carious or erosive lesions, and marked tooth wear)
and did not violate the exclusion criteria (with systemic diseases, users of orthodontic appliance, pregnancy, breastfeeding, having used fluoride compounds in the 2 months prior to collection, having undergone radiotherapy and chemotherapy, practicing aquatic activities in a chlorinated pool) participated in the study\textsuperscript{22}.

Palatal intraoral devices were made of acrylic resin from the plaster model of the upper arch of volunteers, being an intraoral device for each phase of the study\textsuperscript{22}. Each of them had two 6 x 6 x 3 mm (length x width x depth) cavities, one on the right and one on the left side to fix a specimen in each of them\textsuperscript{22}. Specimens were fixed with wax and adapted to the device resin surface level\textsuperscript{24} and on them segments of CrNi 0.6 mm orthodontic wire were carefully and transversely fixed, without coming in contact with the specimens in order to avoid the contact of the tongue with the surface of specimens\textsuperscript{24}. In addition, volunteers were instructed not to touch the specimens with their tongue.

Seven days before and during the experimental period, oral hygiene procedures were standardized\textsuperscript{22}, and volunteers used the same apparatuses: toothbrush (Colgate Classic Clean Média, Colgate-Palmolive Industrial Ltda., São Paulo, Brazil), fluoride dentifrice (Colgate Total 12\textsuperscript{®}, 1,250 ppm F, Colgate-Palmolive Industrial Ltda., São Paulo, Brazil) and dental floss (Hillo, Aperídio Ind. Com. and Rep. Ltda., Aperibe, Rio de Janeiro, RJ, Brazil).

The three phases of the study were interspersed with a 7-day washout period. At each phase, the volunteer was instructed to wait one hour after oral hygiene procedures and insert the intraoral device containing previously eroded specimens. After 2 hours of use, ensuring the formation of the acquired film, intraoral application of the paste supplied in an amount of the size of a pea grain was made in each specimen. Application followed the manufacturer’s recommendations; however, the application was only topical, i.e., without friction, ensuring that the softened surface and the acquired film were not removed.

After 3 minutes of application, intraoral devices were washed with mineral water (Crystal Águas do Nordeste Ltda., Fluorides 0.04 mg / L, pH 6.21, Maceió, AL, Brazil) and reinserted in the mouth only after 3 hours of use (paste substantivity time) \textsuperscript{[25]}, being removed, wrapped in gauze soaked in mineral water (Crystal Águas do Nordeste Ltda., Fluorides 0.04 mg / L, pH 6.2, Maceió, AL, Brazil) and returned to the researcher.

For the evaluation of surface hardness after remineralization (SH\textsubscript{re}), the same microdurometer with the same specifications was used, at 100 \textmu m of post-erosion indentations. The average hardness (post-erosion hardness and post-remineralization hardness) was used to evaluate the hardness recovery percentage (%) SHR according to the following formula: [\text{(final hardness – post-erosion hardness) / (initial hardness – post-erosion hardness)}] \times 100.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SH\textsubscript{des} - KgF/mm\textsuperscript{2}</th>
<th>SH\textsubscript{re} - KgF/mm\textsuperscript{2}</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Placebo (n=10)</td>
<td>306.40 (±37.44)</td>
<td>316.98 (±41.34)</td>
<td>0.006</td>
</tr>
<tr>
<td>G2 CPP-ACP (n=12)</td>
<td>299.24 (±20.03)</td>
<td>336.55 (±22.03)</td>
<td>0.003</td>
</tr>
<tr>
<td>G3 CPP-ACPF (n=12)</td>
<td>295.19 (±50.55)</td>
<td>314.29 (±45.96)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

* One-way ANOVA.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hardness Recovery</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Placebo (n=10)</td>
<td>10.58 (±9.42)</td>
<td>3.19 (±2.72)</td>
</tr>
<tr>
<td>G2 CPP-ACP (n=12)</td>
<td>37.31 (±33.53)</td>
<td>10.64 (±9.12)</td>
</tr>
<tr>
<td>G3 CPP-ACPF (n=12)</td>
<td>19.10 (±19.90)</td>
<td>6.29 (±7.20)</td>
</tr>
</tbody>
</table>

* One-way ANOVA.
Statistical Analysis

Data normality was checked by means of the Shapiro-Wilk test, also using the t-test and One-way ANOVA. The significance level adopted was 5% and the SPSS software version 20.0 was used (SPSS Inc., Chicago, Illinois, USA).

Ethical Aspects

This in-situ study was approved by the local Institutional Ethics Research Committee (Protocol No. 5770916.4.0000.5187) and followed the guidelines of the Declaration of Helsinki.

RESULTS

Final and post-erosion hardness averages were statistically significant for the three pastes tested (Table 1), but with no difference between them (Table 2).

DISCUSSION

Dental caries and erosion are two major dental problems in children and adolescents. Among the various types of treatment for dental erosion, phosphopeptide casein nanocomplex stabilized by amorphous calcium phosphate was postulated as a remineralizing agent of dental structure due to its proven ability to increase salivary levels of calcium and phosphate.

CPP-ACP can be made available in different types of vehicles, notably chewing gum and topical application paste. However, other forms are also studied, as in solution and added to beverage compositions. However, the use of paste as a bioavailability vehicle for CPP-ACP seems to be the best form, since its concentration is higher in this formulation (7.5% in the mousse of CPP-ACP to 10% and the mousse of 10% fluorinated CPP-ACP + 900 ppm fluoride), and the direct contact allowed by topical application probably favors its action.

Most studies on the use of CPP-ACP pastes in the control of dental erosion have been conducted in strictly laboratory approaches, where saliva and acquired film, which are crucial elements in erosive dynamics, are not present. Some studies have shown promising results with the use of CPP-ACP-based pastes.

In the present study, the three pastes used resulted in significant optimization in the re-hardening process, compared to the initial period. However, no difference was observed between CPP-ACP, CPP-ACPF and control pastes. Possibly, this finding is a result of the salivary effect, which, as already established in literature, plays an important role in the reduction of tooth wear in the face of an erosive / abrasive attack. In addition to being able to protect the dental structure from erosion through its buffering capacity, washing and demineralization inhibition, saliva also has the potential to re-harden the eroded enamel, providing calcium, phosphate and fluoride necessary for mineral precipitation.

It should be emphasized that this study followed recommendations present in literature, resembling natural clinical situation, and, therefore, provides information pertinent to the reality of the oral environment. However, for being a
short-duration test, it cannot be inferred about the effectiveness of the tested groups regarding more advanced erosion stages. Therefore, it is suggested that the action of CPP-ACP and CPP-ACPF pastes on erosive wear should be evaluated in erosive cycling studies.

CONCLUSION

CPP-ACP pastes, supplemented or not by fluoride, did not demonstrate higher efficacy in re-hardening the eroded enamel compared to placebo paste.

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