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Histidine buffering of acidic fluoridated solutions increases CaF_2-like formation on carious enamel

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Aim: Since the reactivity of fluoride with enamel decreases when the pH of slightly acidic solutions is not maintained, we evaluated the effect of acidic fluoridated solutions buffered with histidine on the formation of loosely (CaF_2-like) bound products on enamel. **Methods:** Demineralized enamel slabs were treated with 0.05% NaF solution at pH levels of 5.0, 5.5, 6.0, and 6.5, either buffered or not with histidine (n=12). CaF_2-like product formation was determined in enamel, and the data were submitted to ANOVA, Tukey, and Dunnett's tests. **Results:** Buffered fluoride solutions, regardless of pH, formed higher concentrations of CaF_2-like products on enamel than the respective group without buffering ($p<0.05$). **Conclusion:** The findings show that the buffering increases CaF_2-like formation on carious enamel, although a direct effect of histidine cannot be entirely ruled out.

Keywords: Fluorides. Dental enamel. Dental caries. Buffers. Histidine.

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Introduction

Fluoride is the most relevant anticaries ion[1], and its effect is considered mainly local. One of the caries control mechanisms comes as fluoridated toothpastes, rinses, and professional application products promoting a chemical reactivity with the enamel and dentine surface[1]. During this reactivity, two fluoridated products are formed: one firmly bound as fluorapatite (FAp) and another loosely bound as a calcium fluoride-like product (CaF_2-like). During the formation of both products (Figure 1)[2], phosphate (PO_4^{3-}) and hydroxyl ions (OH^-) are released from the hydroxyapatite crystals into the environment (liquid/solid interface). As these two ions act as conjugate bases and accept protons (H^+) from the medium, an increase in local pH is expected (reactions are represented in Figure 1)[2]. The formation of FAp is predominant at low fluoride concentrations (<10 µg F/mL) and occurs mainly by ionic exchange between OH^- ions present in the mineral structure of enamel and fluoride present in the chemical solution[2]. When fluoride is present at high concentrations (>50 µg F/mL), its reaction with enamel involves enamel dissolution, the release of calcium ions, and the subsequent precipitation of CaF_2-like material on the enamel surface[3]. Thus, it is considered that CaF_2-like products have better anticaries properties than FAp[4].

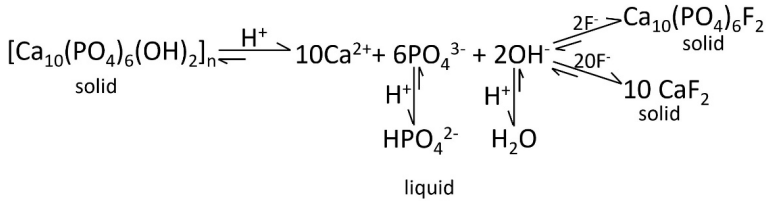

Figure 1. Fluoride reaction with the enamel and relation with the pH of the solution (H^+).

Therefore, the maintenance of pH during fluoride reaction with enamel may be relevant mainly to the formation of CaF_2-like products and commercial fluoride rinses present pH values from 4.23 up to 7.34[5]. If the pH levels are not maintained and increase, the availability of calcium will decrease (Figure 1), diminishing the reaction potential of mineral by-products on enamel. Indeed, it was possible to demonstrate that the pH of fluoridated solutions increases after a reaction with enamel, either sound or with caries-like lesions. In addition, the solution that exhibited the smaller rise in pH during the treatment was the more reactive[6]. In this context, histidine would be a suitable buffer, as its pKa is close to 6, providing buffering capacity within the pH range of 5 to 7. Thus, we aimed to evaluate whether the buffering of slightly acidic fluoride solutions with histidine would result in greater CaF_2-like formation on enamel presenting caries-like lesions.

Material and Methods

Experimental Design

In this in vitro, operator-blind experiment, demineralized enamel slabs (n = 12/group) were randomized into the following treatments: 0.05% NaF solutions at pH 5.0, 5.5, 6.0, and 6.5 either buffered with 0.1 M histidine or not, but with their pH adjusted. As a control group, 0.1 M histidine solution without pH adjustment was used. Histidine concentration was determined by a pilot study. The enamel slabs were subjected to the treatment groups, and loosely bound fluoride formed was determined in enamel as described further.

Fluoride solutions preparation

Fluoride solutions were prepared using NaF (Synth, Diadema, Brazil) at a concentration of 226 ppm F. The buffered solutions were prepared with histidine (L-histidine, Dinâmica, Indaiatuba, Brazil) at a concentration of 0.1 M, and the pH of both the buffered and unbuffered solutions was adjusted with 0.1 M HCl or 0.1 M NaOH. The pH values of the solutions were adjusted using a pH-electrode coupled to a potentiometer, which was calibrated with standard buffers pH 4.0 and 7.0. Fluoride concentration in the solutions was checked as described further.

Preparation of the demineralized enamel slabs

Enamel slabs (4×4×2 mm) were obtained from sound bovine incisor teeth. The dentine was flattened, and the enamel surface was flattened and polished. The dental slabs were then measured with a digital caliper, and the exposed enamel area (mm^2) was determined. All surfaces of the slabs, except the enamel surface, were protected with acid-resistant nail varnish. Caries-like lesions were produced by immersing the dental slabs in a demineralizing solution (0.1 M acetate buffer pH 5.0, 1.28 mM Ca, 0.74 mM $P_{i,}$ and 0.03 mg F/mL) at a proportion of 2 mL/mm^2 enamel for 16 h at 37°C[7].

Reactivity tests

The demineralized enamel slabs were individually immersed in the respective treatment solution at 2 mL/mm^2 of exposed enamel surface under agitation (100 rpm) at room temperature for 10 min, using a validated protocol[8]. After the reaction, the pH of the solutions was again determined, and the variation of pH (ΔpH) was calculated considering $\Delta pH = pH_{initial} - pH_{final}$.

Determination of CaF_2-like product formed

For the extraction of the CaF_2-like product, the slabs were individually immersed in 0.5 mL of 1.0 M KOH solution at room temperature and gently agitated for 24 h[9]. The extract was then neutralized and buffered with 0.5 mL of TISAB II containing 1.0 M HCl[10]. Fluoride in the alkali extract was analyzed with an ion-specific electrode coupled to an ion analyzer as described below.

Fluoride Analysis

Fluoride in the treatment solutions and fluoride in the alkali (CaF_2-like) extract were determined with an ion-specific electrode (Orion 96-06) coupled to an ion analyzer (Orion EA-940; Orion Research) using the direct technique. Calibration curves with standard fluoride solutions (Orion 940907) were prepared and the data were plotted in an Excel spreadsheet (Microsoft). For the analysis of fluoride in the treatment solutions, standards ranging from 2.0 to 32.0 µg F/mL in TISAB II 50% (v/v) were used. For CaF_2-like, standards containing from 0.125 to 16.0 µg F/mL in 0.5 M KOH and TISAB II (1 M HCl) 50% (v/v) were used. From the amount of fluoride in the alkali extract, the concentrations of CaF_2-like in enamel were calculated and expressed in µg F/cm^2.

Statistical Analysis

After exploratory analysis using SAS software (SAS Institute Inc., Cary, NC, USA, Release 9.1, 2003), the ΔpH was analyzed using Kruskal-Wallis, Dunn, and Mann-Whitney tests. The values of CaF_2-like concentration were transformed by the square root. The data were subjected to analysis using the ANOVA test in 4 × 2 + 1 (pH × buffer + control) factorial design, followed by Tukey's test for comparisons among groups and Dunnett's test to compare the experimental groups with control, at a 5% level of significance.

Results

The pH of the treatment solutions measured after the reaction with the enamel slabs (Table 1) showed that histidine was effective in preventing pH changes in the solutions at pH 5.0, 5.5, and 6.0. For pH 5.5 and 6.0, the pH increased 0.12-0.13 units in the fluoridated solutions without histidine. When comparing the solutions with and without histidine, statistically significant differences were found in the groups at pH 5.0 and 6.0 ($p<0.05$).

Table 1. Median (minimum; maximum) of ΔpH values according to the treatment with F solutions, buffered or not, at different pH values (n = 12).

pH	Without histidine	With histidine
5.0	0.04 (-0.11; 0.08) Ab	-0.14 (-0.18; -0.07) Bb
5.5	0.12 (0.02; 0.24) Aa	-0.10 (-0.16; 0.02) Ab
6.0	0.13 (0.07; 0.25) Aa	0.01 (-0.03; 0.07) Ba
6.5	-0.07 (-0.19; 0.07) Ab	-0.09 (-0.18; -0.02) Ab

Medians followed by distinct letters (uppercase in rows and lowercase in columns) are different according to the Kruskal-Wallis, Dunn, and Mann-Whitney tests (p<0.05).

Concerning CaF_2-like formation (Table 2), the effects of the isolated factors, pH ($p<0.0001$), buffer ($p<0.0001$), and the interaction of the factors ($p=0.0006$) were significant. Dental slabs from all groups treated with buffered solutions showed higher CaF_2-like concentrations than those without histidine ($p<0.0001$). In the groups without buffer, there was no significant difference between pH 5.0, 5.5, and 6.0 ($p>0.05$).

Considering the groups with buffer, the slabs treated with F solution at pH 5.0 had the highest concentration of CaF_2-like, which statistically differed from all other groups ($p<0.0001$). All groups statistically differed from the control group ($p<0.05$).

Table 2. Mean (standard deviation) of the CaF_2-like (µg F/cm²) products found on enamel according to the treatments and the control (n=12/group).

pH	Without histidine	With histidine
5.0	4.80 (1.23) Aab	14.36 (3.34) Ba#
5.5	4.90 (1.12) Aab	9.91 (2.20) Bb
6.0	5.20 (1.27) Aa	10.04 (1.33) Bb
6.5	3.59 (1.19) Ab	8.89 (1.20) Bb

Control (histidine solution): mean = 0.21, standard deviation = 0.04. All groups differed from the control group according to Dunnett's test ($p<0.05$). Distinct letters (uppercase in rows and lowercase in columns) indicate statistically significant differences ($p<0.05$). The # represents an outlier statistically identified in the group (n=11).

Discussion

This initial hypothesis that the buffering of 226 ppm F solutions slightly acidic (pH ≥ 5.0) would increase the formation of CaF_2-like products on enamel was accepted. Histidine was chosen because the pKa of its imidazole group is 6.0, providing good buffer capacity to prevent the pH increase that occurs when fluoride solutions at pH levels between 5.0 to 6.0 react with enamel[6]. Based on Table 1, the histidine buffer effectively maintained pH stability, while solutions with initial pH values of 5.0, 5.5, and 6.0 exhibited a slight pH increase after exposure in the absence of histidine, as expected and shown in Figure 1. Consequently, an increased formation of CaF_2-like products was observed in all fluoridated groups containing histidine, especially at pH 5.0.

The maintenance of lower pH values may have prevented chemical equilibrium from being reached[11], sustaining hydroxyapatite dissolution and releasing Ca^{2+} for the formation of CaF_2 products. The pH of the solutions played a crucial role during the reaction with the dental substrate, as H^+ ions could potentially interfere with fluoride reactions. Specifically, for the solution with pH=5.0 (Table 2), the increase in fluoride concentrations could stem from either the easier diffusion of HF or from the dissolution and recrystallization of enamel, particularly potentiated under low pH conditions[2,3]. Moreover, in enamel with caries-like lesions, the formation of CaF_2-like compounds could serve as a local ion reservoir, increasing F bioavailability to the enamel[1,4]. Considering the action mechanism of fluoride, under undersaturated conditions, fluoride ions will be released from CaF_2-like products, thereby promoting remineralization or decreasing demineralization[1,4,12].

Previous studies have reported more effective treatments and an increase in the FAp and CaF_2-like formations when products with acidic pH were evaluated, particularly with more concentrated fluoridated solutions, professional gels, or toothpastes[13,14]. In the present investigation, the most acidic solution had a pH of 5.0, and the concentration of fluoride in the solution was lower (226 ppm), thus, the increase in the formed fluoride products cannot be solely attributed to Ca^{2+} release induced by

the low pH. Corroboratively, an increase in CaF_2-like products was observed for the histidine-NaF group at pH 6.5 (Table 2), suggesting a specific effect mediated by histidine. L-histidine may facilitate the nucleation and precipitation of hydroxyapatite[15] due to its ability to chelate calcium ions or increase local concentrations of PO_4^{3-} and Ca^{2+}, which could then reprecipitate as CaF_2-like compounds. Future investigations should explore other reactions occurring in the presence of histidine in fluoridated solutions, including its potential role in the formation of fluoride-enriched mineral complexes or the transport of fluoride into caries-like enamel.

The limitations of the study include the use of an in vitro caries model that did not use biofilm or pH cycling, the non-determination of FAp products, and the inability to extrapolate the subclinical and clinical effects of increased reactivity of the fluoridated solutions. It is also imperative to consider that the effects observed in the present study may be related to the chemical structure of histidine rather than solely to its buffering capacity.

In conclusion, the addition of histidine as a buffer to fluoridated solution has the potential to enhance fluoride reactivity on enamel with caries-like lesions.

Data availability

Data is available on demand from referees.

Conflict of Interest

The authors have no conflicts of interest to declare.

Author Contribution:

Waldemir Francisco Vieira-Junior: Data curation, investigation, methodology, original draft, writing, review, and editing. **Lenita Marangoni Lopes:** Data curation, investigation, methodology, review, and editing. **Jaime Aparecido Cury:** Conceptualization, data curation, investigation, methodology, writing, review, and editing. **Cinthia Pereira Machado Tabchoury:** Conceptualization, data curation, investigation, methodology, project administration, validation, original draft, writing, review, and editing. All authors actively participated in the discussion of the manuscript's findings and have revised and approved the final version of the manuscript.

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