Evaluation of commercial fluoride solutions on bovine enamel demineralization, using pH-cycling model

Avaliação de soluções fluoretadas comerciais na redução da perda mineral em esmalte bovino, utilizando modelo de ciclagem de pH

Abstract

Purpose: The aim of this in vitro study was to evaluate the fluoride concentration in mouthrinses and their capacity to intervene with the caries process.

Methods: The analysis of the fluoride concentration in the solutions was carried with ion specific electrode connected to an ion analyzer. Bovine incisors enamel blocks with previously known superficial microhardness were submitted to a pH cycling model and treated with fluoridated solutions, and later reanalyzed for microhardness and fluoride incorporation.

Results: The fluoride concentration (ppm) found in the solutions SANiFill- Sanikids®, Colgate Plax Fresh Mint®, Oral B®, Sorriso Fresh® and Cepacol® (not fluoridated) were, respectively: 242.91±21.89; 248.42±3.55; 248.29±11.31; 257.61±17.57 and 35.37±10.64. The lowest loss of superficial microhardness was observed with the use Sorriso Fresh® (29.66%±8.84), followed by Plax Fresh mint® (32.99%±14.09) and Oral B® (43.00%±18.34).

Conclusion: The data suggest that fluoride solutions, in proper concentrations, are capable of intervening on the phenomena of demineralization and remineralization, promoting fluoride incorporation and, consequently, decreasing the loss of microhardness on dental enamel.

Key words: Microhardness; fluoride; pH-cycling

Resumo

Objetivo: O objetivo deste estudo in vitro foi analisar a concentração de flúor em diferentes soluções fluoretadas para bochechos e sua capacidade de interferir no processo de cárie.

Metodologia: A análise da concentração de flúor nas soluções para bochecho foi realizada com eletrodo específico para íon flúor acoplado a um analisador de íons Orion. Blocos de esmalte de dentes incisivos bovinos com a microdureza superficial conhecida foram submetidos à ciclagem de pH e uso de soluções fluoretadas, sendo, posteriormente, avaliados por uma nova análise de microdureza e também pela incorporação de flúor.

Resultados: As concentrações de flúor (ppm) encontradas nas soluções SANiFill®, Colgate® Plax®, Oral B®, Sorriso® e Cepacol® (não fluoretada) foram, respectivamente: 245,94; 247,61; 251,50; 258,01 e 38,44. A menor perda de porcentagem de dureza superficial foi observada com o uso da solução fluoretada Sorriso® (29,7%±14,4), seguida por Colgate® Plax® (33%±14,1) e Oral B® (43%±18,3). Todas as soluções fluoretadas analisadas mostraram capacidade de incorporar flúor no esmalte dental.

Conclusão: Os dados sugerem que as soluções fluoretadas foram capazes de interferir nos fenômenos de desmineralização e remineralização, promover incorporação de flúor e, consequentemente, diminuir a perda de dureza no esmalte dental.

Palavras-chave: Microdureza; flúor; ciclagem de pH
Introduction

Researches on the effect of fluoride on oral health began more than 100 years ago, but it was on the second half of the 20th century that the focus of the studies was the development and evaluation of fluoride products (1). Since then, the frequent use of composites with low fluoride concentration, such as dentifrices, has shown efficiency on the control of dental caries (2), by its constant presence on the oral cavity, thus interfering on the phenomena of de- and remineralization on the interface tooth-biofilm (3).

On the other side, the use of fluoride mouthrinses has been suggested as an additional prevention method, of individual character, in terms of risk or caries activity, or may be important in a collective sense considering the prevalence of dental caries on the population, or groups epidemiologically vulnerable (4,5).

In order to interfere on the dynamics of caries development, fluoride solutions must be in the adequate concentration and thus be able to react with dental tissues. In an attempt to regulate the manufacturing of such products in Brazil, the National Sanitary Surveillance System (ANVISA – Agência Nacional de Vigilância Sanitária) established, by Resolution 29 on August 2000, the concentration of fluoride in solutions at 225 ppm, with variation of 10% (6).

The purpose of this study was to evaluate the anti-caries performance of four commercial fluoride solutions available in the Brazilian market, using a pH cycling model and bovine enamel, through analysis of microhardness and determination of fluoride concentration on dental enamel.

Methods

Experimental Design

A blind in vitro study was carried to verify the ability of fluoride solutions to inhibit demineralization of enamel. Enamel blocks (4 x 4 mm) obtained from bovine incisors were polished sequentially and selected according to their surface microhardness (MSI, n=50). The blocks were submitted to pH cycling and treatment with fours commercial fluoride solutions and one solution without fluoride (negative control).

After cycling, surface microhardness was analyzed, and both loss of surface hardness (%PDS) and fluoride concentration on enamel (µg F/mm²) were calculated. For data analysis, mouthrinses, and variables MSI, MSF, %PDS and fluoride concentration on enamel (µg/mm²) were considered as variation factor.

Fluoride concentration analysis on mouthrinses solutions

The analyzed solutions were: SANiFill-Sani Kids® (Facilit Odontologia e Perfumaria LTDA – Rio de Janeiro, RJ, Brazil), Colgate Plax® Fresh Mint (Colgate-Palmolive Ind. e Com. LTDA – São Paulo, SP, Brazil), Oral B® (Procter & Gamble do Brasil LTDA – São Paulo, SP, Brazil), Sorriso® Fresh (Colgate-Palmolive Ind. e Com. LTDA – São Paulo, SP, Brazil) e Cepacol® (SanoFi-Aventis Farmacêutica LTDA – Suzano, São Paulo, Brazil), the latter is a non-fluoridated solution used as negative control. The solutions were coded as S1, S2, S3, S4, CN, respectively. Solutions, in triplicate, were numbered from 1 to 15; 1 mL of each solution was inserted with a pipette in flasks. The flask was completed with deionizer water until 100 mL, thus obtaining three dilutions for each product (7). Previously to the analysis of the samples, a calibration curve was carried, with patterns from 0.1 to 1.0 ppm F⁻ obtained from Fluoride Standard (100 ppm F⁻) – Orion Ion Plus® (Thermo Fisher Scientific Inc. – Waltham, MA, USA). The fluoride in the solutions was determined by mixing 1mL of the diluted samples with 1mL of TISAB II (acetate buffer at 0.75 M, pH 5.0, NaCl 1.0 M and CDTA 0.4%), under agitation. Fluoride analysis was performed using a specific electrode for ions Fluoride (Orion 96-09 – Orion Research Inc. – Boston, MA, USA) connected to an ion analyzer (Orion 720-A – Orion Research Inc. – Boston, MA, USA).

Preparation of dental blocks and determination of enamel surface microhardness

Fifty enamel blocks (4 x 4 mm) were prepared from bovine incisors. The enamel surface was ground flat with abrasive papers 600 and 1200 grit under water and polished with felt paper wet by diamond suspension (Diamond Suspension 1 Micron – Water Base nº 406530 – Buehler® Metadi®, Lake Bluff, IL, USA).

Initial surface microhardness (MSI) of the enamel block was measured with a microhardness tester (Shimadzu HMV-2000 – Shimadzu Corporation, Japan) with Knoop indenter. A referential indentation on the base line was created using a 100 g weight for 5 seconds. In addition, five indentations with 100 µm space from each other were prepared using a 50 g weight for 5 seconds, and from these, an average in Knoop (KHN) was obtained (8). Only blocks with average surface hardness 340 KHN (±10%, between 316 and 374 KHN) were used, and those samples were randomly assigned into 5 groups.

pH cycling and treatments

The enamel blocks were submitted to pH cycling for 5 days, simulating high caries challenge, according to Featherstone et al. (10). The groups of blocks were kept in 50 ml of demineralizing solution with pH 4.3 (2.0 mM calcium, 2.0 mM phosphate in acetate buffer 0.075 M) at 37˚C for 3 hours; and in 50 mL remineralizing solution with pH 7.0 (1.5 mM Ca, 0.9 mM PO₄²⁻, 150 mM KCl in Tris buffer 0.1 M) at 37˚C for 20 hours (11).

During pH cycle, the enamel blocks were treated twice a day for one minute with 50 mL of the solutions in analysis (before and after demineralization cycle). The negative control group was treated with a non-fluoridated solution and the remaining groups with the experimental fluoride solutions (0.05% NaF). After 5-day cycle, the blocks were immersed for 48 hours in remineralizing solution and then the superficial microhardness was reevaluated, as well as the presence of fluoride on enamel.
Fluoride solutions and enamel demineralization

Post-treatment microhardness analysis of enamel surface

After pH cycling, MSF of enamel block was again measured; ten indentation (5 above and 5 below base line) were created and a new average value (KHN) was found, and the percentage of surface hardness loss was calculated [%PDS=100 (MSF – MSI)/MSI].

Determination of fluoride concentration on enamel

Dental blocks were protected by acid-resistant varnish, allowing only the enamel surface free. Then, a layer of enamel was removed from each block by immersion in 0.5 mL of HCl at 0.5 M for 30 seconds under agitation (12). An equal volume of TISAB II (pH 5.0), modified with NaOH at 20 g/L, was added to each flask to neutralize the reaction. Fluoride measurements were carried using specific fluoride electrode and ion analyzer, previously calibrated with standard fluoride solutions from 0.1 to 1.0 ppm F. The width of the removed enamel layer was calculated from the concentration of inorganic phosphorus, determined by colorimetric method of Fiske and Subarrow (13); rate of phosphorus on enamel of 17.4% and density 2.92 were considered (9).

Statistical analysis

Statistical analysis was performed using the software Epi-Info 3.5.1 for Windows, with significance level of 5%. Data were submitted to normality and homogeneity tests, Shapiro-Wilk’s and Bartlett’s, respectively. Variables MSI, MSF, %PDS and µg F/mm² showed heterogeneity and were submitted to Kruskal-Wallis test followed by Mann-Whitney test for two-tailed comparison (14,15).

Results

Fluoride concentration on solutions showed superior values to those related by the manufacturer (Table 1). Table 2 shows that after demineralization-remineralization and use of solutions all groups presented loss of superficial hardness and differed significantly (P<0.001) from control group. Highest and lowest losses of hardness were observed with solutions S1 and S4, respectively (Table 2). Regarding fluoride present on enamel, all groups presented statistically significant difference (P=0.001) when compared to control group (Table 1).

Discussion

The use of fluoridated mouthrinses represents yet another alternative to maintain fluoride in the oral cavity and interfere on the dynamics of caries development. The sine-qua-non condition for commercial products to have anticaries potential is that they should present a significant concentration of soluble fluoride. The results obtained in this study show that the analyzed solution are above the values specified by the manufacturer. Solutions S1 and S2 presented values according to Resolution 29 of ANVISA (6), which establishes a 225 ppm fluoride concentration, with 10% variation. Solutions S3 and S4 presented value above that specified by ANVISA. The concentration specified by the resolution is coherent to the values stated by literature as efficient; the results of several studies support the concept that the frequent use of relatively low concentration fluoride would be the most appropriate way to control the development of dental caries (16). Therefore, the solutions tested that presented fluoride concentration around 225 ppm can, regarding the dose prescribed, potentially present efficiency if used on the recommended frequency and for an adequate period of time.

The results of enamel surface microhardness analysis showed that fluoridated solutions were capable of reducing mineral loss, even during cariogenic challenging situations (pH cycling), when compared to a non-fluoridated solution (CN), which is in agreement to developed studies with similar methodology (17). The differences found on percentage of hardness loss (%PDS) from S1 to S2 and S4, may be related to other components not evaluated in this study; although this has not been observed in other study (18) that evaluated whether the presence of composite cetylpyridinium chloride (antiseptic) would reduce the effect of fluoride on caries development. The results obtained in the present study confirm the action of fluoride on dynamic development of caries, resulting from unbalance between phenomenon of de- and remineralization of hard dental tissues (19,20).

Table 1. Concentration (Mean ± SD; n=10) of fluoride (F) on mouthrinses solutions (as displayed on packaging) and present on enamel after pH cycling.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Analysis ppm F on solutions</th>
<th>µg F/mm² on enamel*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>242.91±21.89 (226)</td>
<td>11424.64±2273.46</td>
</tr>
<tr>
<td>S2</td>
<td>248.42±3.55 (225)</td>
<td>12595.58±6823.82</td>
</tr>
<tr>
<td>S3</td>
<td>248.29±11.31 (226)</td>
<td>9226.56±2009.99</td>
</tr>
<tr>
<td>S4</td>
<td>257.61±17.57 (225)</td>
<td>9303.27±2373.27</td>
</tr>
<tr>
<td>CN</td>
<td>35.37±10.64 (not shown)</td>
<td>96.85±48.66</td>
</tr>
</tbody>
</table>

* Different letters indicate statistical difference between groups for each analysis (Kruskal-Wallis, P<0.001).

Table 2. Values (Mean ± SD, n=10) of microhardness (Knoop) of surface (MSI and MSF) before and after pH cycling, according to analysis and groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MSI</th>
<th>MSF*</th>
<th>%PDS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>351.7±18.29</td>
<td>168.6±54.95</td>
<td>52.41±4.38</td>
</tr>
<tr>
<td>S2</td>
<td>347.5±17.04</td>
<td>232.8±49.04</td>
<td>32.99±14.09</td>
</tr>
<tr>
<td>S3</td>
<td>352.9±16.43</td>
<td>200.5±64.76</td>
<td>43.00±18.34</td>
</tr>
<tr>
<td>S4</td>
<td>348.3±23.70</td>
<td>246.4±43.76</td>
<td>29.66±8.71</td>
</tr>
<tr>
<td>CN</td>
<td>311.6±90.18</td>
<td>27.0±19.53</td>
<td>89.61±8.71</td>
</tr>
</tbody>
</table>

* Different letters indicate statistical difference between groups for each analysis (Kruskal-Wallis, P<0.001)

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Fluoride concentration on sound enamel is generally between 20 and 100 ppm, depending on fluoride ingestion during dental development, however the mineral incorporated to the tooth is insufficient to alter enamel solubility facing pH variation (21); only when fluoride is incorporated to a new crystal formed on the surface during remineralization a higher resistance to acid attack is observed (21,22). The present results show that all solutions tested have ability to incorporate fluoride on dental enamel, with concentration significantly higher than the non-fluoride solution, which strongly suggests its influence on lessening the loss of hardness, corroborating with previous studies (19,22,24).

Conclusions
The analyzed fluoride solutions, when in adequate concentration, showed not only the capacity of incorporating fluoride on dental enamel, as well as of interfering on the dynamics of development of dental caries, reducing demineralization and activating remineralization of dental enamel.

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References