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In Vitro Erosive Effect of Pediatric Medicines on Deciduous Tooth Enamel

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This study evaluated, in vitro, the erosive potential of pediatric liquid medicines in primary tooth enamel, depending on the exposure time. Sixty deciduous incisors were randomly assigned to 4 groups (n=15), according to the immersion solutions: guaifenesin; ferrous sulfate; salbutamol sulfate and artificial saliva. The immersion cycles in the medicines were undertaken under a 1-min agitation, which wasperformed three times daily, during 28 days. Surface microhardness was measured at 7,14, 21 and 28 days. The titratable acidity and buffering capacity of the immersion media were determined. Data were analyzed by Analysis of Variance and Tukey's test (α=0.05). Salbutamol sulfate caused a gradual loss in enamel microhardness deciduous, observed at all times ($p<0.005$). Exposure to guaifenesin or ferrous sulfate resulted in significant decrease of enamel microhardness only after 28 days ($p<0.005$). In the control group (artificial saliva), microhardness did not changed ($p>0.005$) at any of the studied times. Scanning Electron Microscopy (SEM) images revealed that after 28 days the surfaces clearly exhibited structural loss, which was unlike those immersed in artificial saliva. Erosion of deciduous enamel was dependent on the type of medicine and exposure time.

Key Words: deciduous teeth, enamel, erosion, medicines.

Introduction

Dental erosion is defined as a progressive loss of dental hard tissues by chemical dissolution without bacterial involvement (1). In modern society, the changing habits have contributed to an increased incidence of dental erosion, especially in children and adolescents (2,3). Erosive tooth wear is a multifactorial irreversible process that may be caused by intrinsic, extrinsic or idiopathic factors (3).

The intrinsic etiologic factors are related to the contact of tooth tissues with stomach acids (i.e., regurgitation and reflux disorders) (4). Increased acidic food and drink consumption has become the primary extrinsic source of dental erosive agents (1), although acidic medicines and behavioral factors have also been identified as extrinsic etiologic factors in dental erosion (5-8).

Liquid oral medicines are usually prescribed for children to aid compliance (7). Acidic preparations are often necessary for drug dispersion, chemical stability maintenance, to ensure physiological compatibility and to improve flavor (6,7). In addition to the acidic components, other factors such as prolonged and frequent ingestion (i.e., two or more times daily), bedtime and between meals consumption, high viscosity and the collateral effect of reduced salivary flow, may contribute to increase the risk for medication-induced dental erosion (1,2,9).

Some *in vitro* researches reported that medications can affect enamel hardness, and cause morphological and roughness alterations (5,9). Nevertheless, the results of these studies are limited to a small number of medicines, and the literature is scarce of articles that investigate the effects of medications on permanent and deciduous tooth enamel (9).

According to some authors (11,12), enamel thickness in deciduous teeth, lower mineralization levels and a lower structural arrangement are the main differences between deciduous tooth enamel compared with that of permanent teeth. However, some controversy remains concerning the susceptibility of deciduous teeth to caries and erosion process compared with permanent teeth (13,14).

Therefore, in view of the increased use of oral medicines by children for prolonged periods in recent years, especially those with chronic diseases, the aim of this study was to assess *in vitro* on deciduous tooth enamel the erosion potential of liquid oral pediatric medicines that are commonly used in pediatric patients to treat disorders such as anemia, asthma, bronchitis and cough.

Material and Methods

This study protocol was approved by the local Ethics Committee under the registration number 2010.1.908.58.6.

Experimental Design

The medications included three experimental treatments: guaifenesin (Vick™ Mel Syrup; Procter & Gamble Higiene e Cosméticos Ltda, Louveira, SP, Brazil); ferrous sulfate (Sulferrol™; Bunker Ind. Farmacêutica Ltda, São Paulo, SP, Brazil); salbutamol sulfate (Laboratório Teuto Brasileiro, Anápolis, GO, Brazil) and one control (i.e.,

artificial saliva) (13); the exposure times were as follows: 0 (baseline), 7, 14, 21 and 28 days. The compositions of the immersion media employed are presented in Table 1. This study was designed as randomized complete blocks (n=15) and comprised 60 specimens. These drugs were chosen because they are syrups that are commonly used in pediatric patients for prolonged periods. Salbutamol sulfate is an antiasthmatic; ferrous sulfate is an iron supplement and/or antianemic, and guaifenesin is an expectorant.

The quantitative response variable was percent surface Knoop microhardness (KHN) in kgf. Scanning electron microscopy (SEM) was used to observe the effects of the medications on deciduous enamel surface morphology.

Tooth Selection

A total of 80 healthy human primary central incisors, recently exfoliated/extracted, were donated by the Human Tooth Bank of Ribeirão Preto School of Dentistry, University of São Paulo, Brazil and were immersed in 0.1% thymol solution at 4 °C for 48 h. Prior to use, the teeth were hand scaled and cleaned with pumice-water slurry using Robinson bristle brushes in a low-speed handpiece. Then, the teeth were examined with a stereomicroscope (Nikon Inc. Instrument Group, Melville, NY, USA) at 10× magnification to discard those with cracks, fractures or structural abnormalities that could interfere in the results.

Selection and Preparation of Specimens

When present, the roots were removed at the cementoenamel junction with a water-cooled diamond saw of a precision sectioning machine (Isomet 1000; Buehler, Lake Bluff, IL, USA). Each crown was fixed with plastic wax in the central orifice of an acrylic plate. The buccal surface was faced upwards using a parallelometer (ElQuip, São Carlos, SP, Brazil) to secure the flattest region of the buccal surface (incisal third) parallel to the plate. Next, the crowns were stabilized with red wax (15).

The specimens had their buccal enamel surfaces flattened with 600 and 1200-grit Al_2O_3 abrasive papers (Buehler Ltd.), polished with 0.3-µm alumina paste (Alpha and Gamma Micropolish; Buehler Ltd.) and felt paper using a water-cooled low-speed polishing machine (Politriz DP-9U2; Struers A/S, Copenhagen, Denmark). The specimens were ultrasonically cleaned in deionized water for 10 min.

The test sites were demarcated by attaching a piece of insulating tape with a 2-mm diameter central hole on each surface. The tooth/plate sets were rendered acid-proof by coating them with 2 layers of cosmetic nail polish. The previously delimited circular area on the flattest region of the buccal surface was left uncoated. Then, the specimens were stored at 37 °C in a 100% relative humidity environment. Prior to the immersion cycles, the specimens were immersed in artificial saliva for 24 h at 37 °C.

Initial KHN was assessed on the uncoated enamel area using a microhardness tester (Shimadzu HMV-2000; Shimadzu Corporation, Kyoto, Japan). Settings for load and penetration were 25 g and 30 s, respectively. Three indentations spaced 100 µm apart were made at the center of the test enamel surface. An average microhardness value was calculated for each specimen and overall mean microhardness value was obtained from all of the averages. The specimens that presented averages 20% higher or lower than the mean value were discarded, as well as those with an individual standard deviation 20% above or below the average, i.e., amongst the 3 penetrations. On the basis of these criteria, 60 specimens were selected for the microhardness test, and their averages were considered as the initial surface microhardness values.

pH Measure and Buffering Capacity

The pH value of the media used for the immersion cycles and the amount of base required to raise the pH to 7.0 (titratable acidity) were measured with a pH meter (An2000; Analion, Ribeirão Preto, SP, Brazil). To measure

Table 1. Composition, pH, titratable acidity and buffering capacity of the solutions used in the present study

Solutions	Composition	pH	Titratable acidity	**Buffering capacity**
Guaifenesin	guaifenesin 200 mg, hydrolyzed sugar, propylene glycol, sodium citrate, sodium carboxymethyl cellulose, anhydrous citric acid, sodium benzoate, polyethylene oxide N.F., polysorbate 60, 4601 sweeting (aspartame and acesulfame K), honey, honey flavoring system, menthol and eucalyptus, purified water.	4.6	267 mmol/L	111.25 mmol/L x pH
Ferrous sulfate	ferrous sulfate anhydrous 25 mg, citric acid, sugar, caramel color, caramel essence, methylparaben, propylene glycol, sodium cyclamate, saccharin, nipazol, nipagin.	3.7	250 mmol/L	73.52 mmol/L x pH
Salbutamol sulfate	salbutamol sulfate 2 mg, sucrose, sodium benzoate, citric acid, ethyl alcohol 96° G.L., artificial strawberry flavor, bordeaux red dye, deionized water.	3.64	347 mmol/L	99.14 mmol/L x pH
Artificial saliva	methylhydroxybenzoate 2.0 g, carboxymethylcellulose 10.0 g, KCl 0.625 g, $MgCl_2.6H_2O$ 0.059 g; $CaCl_2.2H_2O$ 0.166 g, K_2HPO_4 0.804 g and KH_2PO_4 0.326 g in 1000 mL of deionized water.	7.0	-	-

titratable acidity, 20 g of each drink or solution was titrated with 0.5 M NaOH in 0.02-mL increments at 25 °C. The buffering capacity (β) was calculated with the following equation: $\beta = -\Delta C/\Delta pH$ in which ΔC is the amount of base used and ΔpH is the change in pH caused by the addition of the base (17).

Immersion Cycles

After the initial microhardness measurements, the test surfaces were randomly assigned according to the immersion media to 4 groups (n=15) as follows: guaifenesin (A); ferrous sulfate (B); salbutamol sulfate (C) and artificial saliva (D).

The following immersion cycling protocol was adopted to simulate a usual number of intakes: the specimens were immersed with the exposed area up for 1 min in 10 mL of the medication, under agitation (30 rpm) by a magnetic stirrer (Fanen, São Paulo, SP, Brazil), 3 times daily with 6-h intervals between the immersion cycles, during 5 days (15 immersion cycles). After each immersion cycle, the specimens were washed with distilled water and maintained in 10 mL of artificial saliva at 37 °C, as described by Mcknight-Hanes and Whitford (14) and modified by Amaechi et al. (13), until the next immersion cycle. In the next 2 days, the specimens were stored in relative humidity (15) and the microhardness was then measured after a week. This process was repeated for 4 weeks, totalizing 60 immersion cycles.

The medicines were replaced before each immersion. The control specimens were kept in artificial saliva during the course of the experiment (28 days) with the solution refreshed daily.

Surface microhardness was tested at 7, 14, 21 and 28 days after the continuous and systematic repetition of the daily immersion cycles.

Statistical Analysis

All statistical procedures were performed using statistical software (STATA 9.1; Stata Corporation, College Station, TX, USA) at a significance level of 5%. The data exhibited a normal and homogeneous distribution; thus, microhardness values were analyzed by ANOVA and Tukey's LSD multiple-comparison test with time and medication as study factors. For surface microhardness data, KHN means were used for the factors of medication and time.

Scanning Electron Microscopy Analysis

SEM analysis was performed at the 28th day of the experiment in five teeth from each group (15). The following protocol was undertaken: the specimens were immersed for 10 min in an ultrasonic cleaner (T-1449-D; Odontobrás Ind. e Com., Ribeirão Preto, SP, Brazil) with distilled water; posterior dehydration in an ascending ethanol series (25, 50, 75, 95 and 100%) was performed; the specimens were mounted on stubs, sputter-coated with gold and analyzed in a scanning electron microscope (Philips XL30 FEG-SEM; Philips Electron Optics, Eindhoven, The Netherlands) at 20 kV. The entire buccal surface of each tooth was scanned, and the most representative images were recorded at 150 and 1500× magnifications. The SEM analysis was intended to provide a visual and illustrative comparison of the specimens, and hence, no statistical analysis was performed.

Results

Surface Microhardness (KHN)

KHN and standard deviations, as a function of exposure time to the medications, are displayed at Table 2.

The time × immersion media interaction demonstrated that salbutamol sulfate produced a significant ($p<0.05$) and gradual loss in surface microhardness at all times with no statistically significant difference ($p>0.05$) between the 7th and 14th day. A statistically significant decrease was observed at 21 days, which was statistically different from the 7th day and remained stable until the 28th day. For the guaifenesin and ferrous sulfate groups, there was significant ($p<0.05$) loss of microhardness, which differed from that of artificial saliva only at 28 days.

pH, Titratable Acidity and Buffering Capacity

The pH values ranged from 3.7 (ferrous sulfate and salbutamol sulfate) to 4.6 (guaifenesin). Salbutamol sulfate exhibited the largest titratable acidity (347 mmol/L) ($p<0.05$) compared with ferrous sulfate and guaifenesin. However, guaifenesin exhibited the highest buffering capacity (111.25 mmol/L × pH) ($p<0.05$), which was similar to that of salbutamol sulfate (99.14 mmol/L × pH) ($p>0.05$) (Table 1).

Scanning Electron Microscopy Analysis (SEM)

SEM micrographs of enamel immersed in the medicines and artificial saliva are presented in Figure 1.

After 28 days, the SEM images of the guaifenesin group displayed an accentuated demineralization of the surface that was more pronounced in the interprismatic region, exposing the enamel prism heads (arrows in Fig. 1A). The specimens exposed to ferrous sulfate and salbutamol sulfate clearly exhibited structural loss. The surface was irregular with small enamel depressions, and the enamel prisms were hardly identifiable (arrows in Figs. 1B and 1C). The control group exhibited no microstructure alterations (Fig. 1D).

Discussion

The present research provided evidence that the studied medicines could potentially erode deciduous tooth enamel after successive immersion cycles. The enamel surfaces

presented a decrease in Knoop microhardness, which resulted from the mineral loss caused by medicine intake. The three evaluated medicines have citric acid in their composition, which results in a low pH.

Acids are added to drug formulations as buffering agents to maintain chemical stability, control tonicity or to ensure physiological compatibility and to enhance flavor, and thereby increase the palatability to children (6). Citric acid is the primary acid used in the oral medicines, and despite being a weak acid, citric acid is a potent erosive agent because of its ability to chelate calcium in hydroxyapatite, which reduces saliva supersaturation and increases the dissolution rate of hydroxyapatite crystals (2,13). Some authors reported that as substance pH decreases, the potential of enamel erosion increases (1,4,18).

Nevertheless, the erosive potential of a substance is not exclusively dependent on pH value and acid type. The erosion potential is also strongly influenced by the following substance features: titratable acidity (the greater the buffering capacity, the longer it takes saliva to neutralize the acid), calcium chelation properties, mineral content and adhesion to the dental surface (2,18).

In the current investigation, salbutamol sulfate exhibited the highest value of titratable acidity, which in addition to the presence of ethyl alcohol in its formulation, could, in part, explain the substantial hardness reduction observed in the salbutamol sulfate group. The buffering capacity of guaifenesin was similar to that of salbutamol sulfate. Therefore, the lower decrease in the microhardness values observed in the guaifenesin group might be associated with its higher initial pH value (4.6), its greater buffering capacity and its higher viscosity, which increases the surface tension and decreases the potential of guaifenesin to cause enamel damage, corroborating with Aykut-Yetkiner et al.

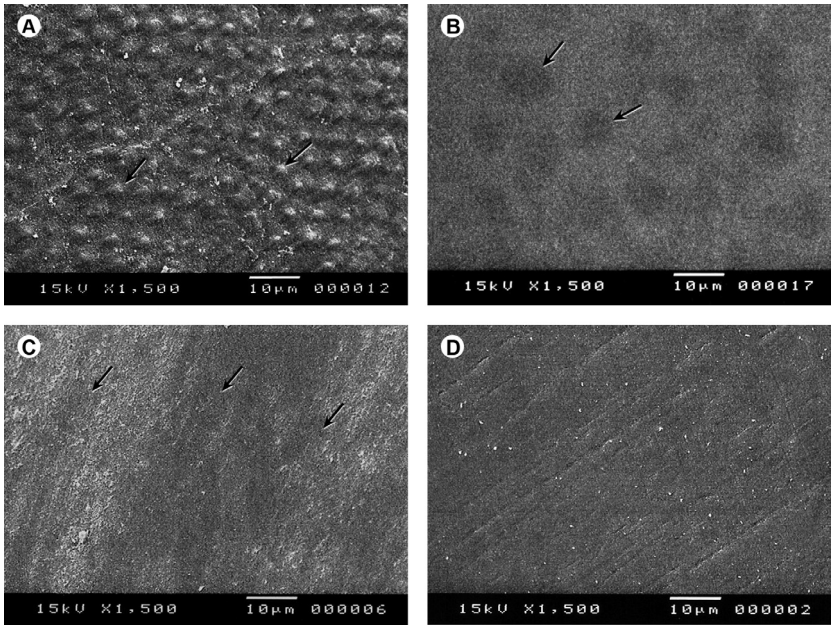

Figure 1. Qualitative analysis of the specimens' enamel surfaces by scanning electron microscopy after 28 days of the experiment. A: guaifenesin; B: ferrous sulfate; C: salbutamol sulfate; D: artificial saliva.

Table 2. Mean values in KHN and microhardness variation before and after the exposure times

Solutions	Time				
	0 (baseline)	7 days	14 days	21 days	28 days
Guaifenesin	310.2 (37.5)Aa	285.2 (29.3)Aab	265.8 (30.5)Aab	251.6 (34.2)Aab	231.6 (43.9)Bb
Ferrous sulfate	306.6 (31.9)Aa	266.2 (43.5)ABa	258.3 (43.8)Aa	246.0 (30.1)Aa	236.7 (50.5)Ba
Salbutamol sulfate	326.6 (28.8)Aa	200.8 (34.6)Bb	160.8 (47.9)Bbc	123.4 (30.2)Bc	118.5 (28.6)Cc
Artificial saliva	304.5 (40.5)Aa	316.5 (39.8)Aa	300.4 (38.4)Aa	301.6 (23.3)Aa	305.7 (31.3)Aa

Capital letters indicate statistical analysis in columns - intergroup comparison. Lowercase letters indicate statistical analysis in lines - intragroup comparison. Different letters indicate statistically significant difference ($p<0.0001$).

(19). According to some studies, buffering properties are important for the erosion potential of a substance because buffering capacity maintains the H^+ ions concentration available for interaction with the enamel surface (2,3,20).

Tooth erosion is defined as a multifactorial process with many risk and protective factors (1,2). In this context, salivary protective mechanisms are considered the most important biological factors during the erosive challenge. Although the use of some medicines, such as antihistamines, and nocturnal administration might reduce salivary flow, citric acid can stimulate salivary flow rates (21). Saliva also has a role in forming the salivary protein-based pellicle on enamel tooth surfaces, which behaves as a diffusion barrier or permselective membrane that prevents direct contact between acids and the tooth surface, thus preventing the demineralization process (2,22). Therefore, in the present study, an artificial saliva medium was used between the erosive immersion cycles because of its proven ability to exert similar remineralizing effect as that of fresh human saliva (22).

Nevertheless, the erosive potential of an acidic challenge might also be related to the frequency and time of acid exposure as well as by the total volume of acid media ingested. In this study it was tried to simulate the usually used dose of these medicines. Some authors speculated that titratable acidity is the preferred predictor of erosive potential during longer erosive challenges and that pH is preferred for short challenges (17,20). Therefore, in this study, the relationship between deciduous tooth erosion and the titratable acidity of the syrup can be due to the employed long periods of erosive challenges.

In the current investigation, the used protocol was based on the following frequency of syrup ingestion: 10 mL taken three times a day, under agitation of the solution during the specimen immersion period. According to some authors, when a substance is ingested, a certain agitation occurs, which favors the substance capacity to cause erosion (20,23).

The experimental period (28 days) was chosen to simulate what would happen over a long treatment. It is likely that longer treatment-induced damages to tooth structures might be greater than those observed in this study. Nevertheless, the syrups used in this study were selected because of their routine use in treating common childhood disorders, such as anemia, asthma, bronchitis and cough.

The method of analysis employed in this study could be used for different (potentially erosive) medicines and saliva (control) at different evaluation periods. According to Shellis et al. (24), microhardness is the most useful method to assess enamel softening. However, considering the results of the salbultamol immersion, it is possible that minerals from artificial saliva storage had been deposited on the enamel surface, and the use of other methods like calcium/phosphate release or profilometry to verify substance loss would be required. Furthermore, it should be emphasized that during microhardness measurements the indentation boundaries were clearly identified.

Other *in vitro* studies observed the erosive effects of some medicines, such as antiasthmatic syrups (9), iron supplements (4,6), antiallergic/expectorant medications (5,6,8) and reported enamel surface roughness, microhardness and morphological alteration findings (9). The erosive potential of ferrous sulfate hypothesized by Passos et al. (25), was confirmed in the present research. However, most of the studies are performed on a permanent tooth substrate. Moreover, no study evaluated the enamel erosive effects of the medications employed in the present investigation. In this manner, Valinoti et al. (9), have reported a reduction of deciduous tooth enamel microhardness analyzing the erosive effects of other acidic medications.

Studies on deciduous tooth substrates are of scientific relevance because structural and morphological differences between deciduous and permanent substrates have been observed (11,12). Furthermore, differences in the chemical composition, rate of formation and ultrastructural appearance between the pellicle on primary and permanent teeth have been reported (12).

Given the findings of this study, clinicians and especially pediatric health professionals and patients should be aware of the risk of erosion during the use of some medicines by children. The knowledge of the erosive potential of these commonly used syrups is mandatory. Erosion in children's teeth may be associated with dental hypersensitivity, loss of the occlusal vertical dimension, eating difficulties, poor esthetics, pulp exposure and abscesses (7). Early diagnosis will help to prevent injuries to permanent teeth. In addition to the risk of dental erosion, frequent exposure to sucrose content in medicines might also increase the risk of caries in children.

This way, oral hygiene or mouth rinsing with water after taking the medication, addition of calcium, fluoride or phosphate to formulations, consumption of the medication at meal times (i.e., not between meals) and use of topical fluoride agents have been recommended to avoid tooth damage that is caused by the regular use of medication (5).

The lack of reported studies that tested the same methodology and materials did not allow for a reliable comparison with published outcomes. Within the limitations of an *in vitro* investigation, the following conclusions can be drawn: the tested medicines decreased deciduous tooth enamel microhardness, which was medication and exposure time dependent; all medicines showed morphological

surface alterations after the experimental period.

Resumo

Este estudo avaliou, *in vitro,* o potencial erosivo de medicamentos líquidos pediátricos em esmalte de dentes decíduos, em função do tempo de exposição. Sessenta incisivos decíduos foram divididos aleatoriamente em 4 grupos (n=15), de acordo com a solução de imersão: guaifenesina, sulfato ferroso, sulfato de salbutamol e saliva artificial. Os ciclos de imersão nos medicamentos foram realizados sob agitação por 1 min, três vezes ao dia, durante 28 dias. As medidas de microdureza superficial foram realizadas após 7, 14, 21 e 28 dias. A acidez titulável e capacidade tampão dos meios de imersão foram determinadas. Os dados foram submetidos à Análise de Variância e teste de Tukey (α=0,05). O sulfato de salbutamol causou uma perda gradual na microdureza do esmalte decíduo, em todos os tempos verificados ($p<0,005$). A exposição à guaifenesina ou ao sulfato ferroso levou à diminuição significante da microdureza do esmalte, apenas após 28 dias ($p<0,005$). No grupo controle (saliva artificial) não houve alteração ($p>0,005$) da microdureza em nenhum dos tempos estudados. As imagens de microscopia eletrônica de varredura (MEV) revelaram que após 28 dias, as superfícies expostas aos medicamentos apresentaram perda estrutural, diferindo dos que foram imersos em saliva artificial. A erosão do esmalte decíduo foi dependente do tipo de medicamento e do tempo de exposição.

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