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In vitro alterations in dental enamel exposed to acidic medicines

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Objective. To evaluate the effect of acidic medicines (Klaricid®, Claritin®, and Dimetapp®) on surface enamel *in vitro*.

Methods. Enamel blocks (n = 104) were randomly distributed into two groups: G1 (pH-cycling simulating physiological oral conditions) and G2 (erosive conditions). Each group was divided into four subgroups, three to be immersed in the medicines and the control in deionized water. Specimen surfaces were evaluated for roughness and hardness at baseline and again after the *in vitro* experimental phase, which included 30 min immersions in the medicines twice daily for 12 days. Scanning electron microscopy (SEM) was also performed after the *in vitro* experimental phase.

Results. All medicines produced a significant reduction in hardness in G1 after 12 days ($P < 0.05$). The three medicines promoted greater roughness after both pH-regimens – G1 and G2 ($P < 0.01$), except for Claritin in G1. Scanning electron microscopy analysis showed erosive patterns in all subgroups. Dimetapp® showed the most erosion and Klaricid® the least, in both groups.

Conclusion. Dimetapp® (lowest pH and viscosity) and deionized water (control) showed the most pronounced erosive patterns. Klaricid® (highest pH and viscosity) presented an *in vitro* protective effect against acid attacks perhaps due to its mineral content and viscosity.

Introduction

Dental erosion is the result of a pathologic, chronic, localized loss of dental hard tissue that is chemically etched away from the tooth surface by acid and/or chelation without bacterial involvement[1]. The aetiology of dental erosion is complex and multi-factorial, and may be either extrinsic or intrinsic[2]. Some intrinsic causes of dental erosion include recurrent vomiting in psychological disorders such as anorexia and bulimia and regurgitation of gastric contents due to gastrointestinal problems[3,4]. Extrinsic sources in children include the regular use of products with low endogenous pH, high titratable acidity, and with a low quantity of calcium, fluoride and phosphate ions[5]. Among these products are acidic foods and drinks[4], and acidic medicines that come in direct contact with teeth, especially if consumed frequently[5–8].

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Liquid oral medicines are usually recommended for sick children for short periods. For chronic diseases, however, they are consumed daily for prolonged periods. Acids are commonly used in medicines as buffering agents to maintain chemical stability, control tonicity or to ensure physiological compatibility and to improve flavor[9], consequently enhancing patient compliance. *In vitro* studies have shown that acidic medicines can reduce enamel hardness of primary teeth[5], influence enamel roughness[10] and cause morphological enamel alterations[11,12], and also induce degradation of composite materials[13]; however, little is known about the effect of oral medicines on tooth surface under erosive conditions.

Therefore, since some children may require frequent use of liquid oral medicines and in some cases these children already present a highly erosive diet by ingestion of acidic foods and drinks, the aim of this *in vitro* study was to evaluate the contribution of acidic medicines to enamel demineralization in a standard pH cycling model and in an erosive model.

Methods

Medicine selection, pH analysis, titratable acidity, concentrations of fluoride, phosphate and calcium and viscosity of the tested media

The choice of paediatric syrup medicines for this study (Claritin®; Schering-Plough, Vila Olímpia, Brazil and Dimetapp Elixir®; Wyeth, São Paulo, Brazil) was based on a previous study[14], which had pointed out that these medicines presented the worst results with regard to pH, titratable acidity and viscosity when taken together. Also the liquid antibiotic was selected based on a previous study[15], which highlighted Klaricid® 50 mg/mL (Abbott, São Paulo, Brazil) from among the 29 antibiotics analyzed, as presenting the worst results with regard to pH, titratable acidity, sugar concentration and viscosity.

Important chemical parameters of the selected medicines were also determined. Fluoride was analyzed using a combined electrode Hach and TISAB III, pH 5.0 (containing 20 g NaOH/I, as a buffer). Phosphorus was determined colorimetrically[16] and calcium was analyzed by atomic absorption spectrophotometry using lanthanum to suppress phosphate interference.

With regard to control, three deionized water samples were analyzed on different days during the experimental phase and a mean of ionic contents for each sample was calculated.

The characteristics of the acidic medicines and deionized water used in this study are shown in Table 1.

Preparation of bovine enamel specimens

Two hundred and fifty enamel blocks (4×4 mm) were prepared from one hundred and twenty-five sound bovine incisors stored in distilled water (pH 6.48 ± 0.12) at room temperature. Crowns were sectioned from the roots and two enamel blocks were obtained from the labial surfaces using an ISOMET Low Speed Saw cutting machine (model no 11-1280-170; Lake Bluff, IL, USA). All enamel blocks were then embedded in acrylic resin, in PVC rings with labial surfaces facing towards the ring base. After resin acrylic polymerization, the sample enamel labial surfaces were wet ground using 600, 800, 1200, 2500 (Norton, São Paulo, Brazil) and 4000-grit abrasive discs (Presi, Grenoble, France) for 10 min each in a water-cooled grinding machine (Panambra DPU-10, Struers; Copenhagen, Denmark) to produce an optically flat area of enamel. After the polishing procedure, samples were viewed under an optical microscope (Aus Jena, model 444181, with a 40 objective; Astro Optics Division, Montpelier, MD, USA) in order to check that the surfaces were flat, polished and free of irregularities that could interfere with the roughness evaluation.

Baseline analysis

Baseline surface roughness of each enamel specimen (*Ra* - μm) was measured using a surface roughness tester (Surftest SJ 201; Mitutoyo Co., Kawasaki, Japan). Three

Table 1. Chemical parameters of the acidic medicines and control.

Characteristics	Klaricid®	Claritin®	Dimetapp Elixir®	Deionized water
Batch number	640470-A	53425	801	—
Active principle	Clarithromycin	Loratadine	Brompheniramine and Pseudoephedrine	—
pH	5.04	2.80	2.70	5.60
Titratable acidity (volume of 0.1 N NaOH, mL)	40.02	14.59	11.96	0.10
Viscosity at 20 s^{-1}(cP)	1660	19.70	13.30	0.65
Presence of citric acid	Yes	Yes	Yes	No
Calcium (μg/mL)	11.16	10.98	9.82	<0.01
Phosphate (μg/mL)	33.32	<1.5	<1.5	<1.5
Fluoride (μg/mL)	0.17	<0.025	<0.025	<0.025

roughness measurements spaced at 60° were recorded for each specimen (cut-off length of 0.25 mm). The mean value of the three measurements was recorded as the baseline surface roughness value for each specimen. Forty enamel blocks were discarded due to their discrepant roughness values leaving a total of 210 specimens with surface roughness of 0.04–0.15 μm for surface microhardness (SMH) evaluation.

To determine initial SMH, a hardness tester (Micromet 2003, model 1600-5300; Buehler, Lake Bluff, IL, USA) was calibrated with a Knoop tip and load of 50g was applied for 15 s. Five indentations spaced 100 μm from each other were made in the centre of the enamel surface and their average value was taken as equivalent to the hardness value of the specimen. One hundred and seven enamel blocks with SMH ranging from 272 to 392.48 KHN (Knoop Hardness Number) were selected for the experimental phase, as these were considered compatible with sound dental bovine enamel. All the selected blocks were stored in a 100% humidity environment. At this point three enamel blocks were set aside for scanning electron microscopy (SEM) evaluation to assess surface topography at baseline.

Experimental protocols

After baseline analysis, enamel blocks were randomly distributed, according to experimental protocols into two different groups (G1 and G2) with two different pH-cycling models. Each group was divided into four subgroups ($n = 13$), one for each media immersion: Klaricid® (A); Claritin® (B); Dimettap® (C) and deionized water (control – D).

The standard pH-cycling model[17] without fluoride (G1) was used to simulate physiological oral conditions (2 h in an acid solution, 21 h in a neutral solution and 1 h immersed in a medicine or control at 37°C), whereas an erosive pH-cycling model (G2) was used to simulate an erosive oral environment (16 h in an acid solution, 7 h in a neutral solution and 1 h immersed in a medicine or control at 37°C). The experimental protocol is shown in Fig. 1. The acid solution contained 3 mmol/L of calcium, 3 mmol/L of phosphate and 50 mL/L of acetic acid in a pH adjusted to 4.5 with NaOH[18], while the neutral solution was composed of 1.54 mmol/L of calcium, 1.54 mmol/L of phosphate, 20 mmol/L of acetic acid, and 0.308 g of ammonium acetate with pH adjusted to 6.8 with potassium chloride at 37°C[18].

The amount of each medicine, deionized water and neutral and acid solution for each group was 20 mL. The medicines and deionized water were replaced before each immersion and the solutions were changed daily. After immersion with a medicine the specimens were rinsed with deionized water. On the 13th day, surface roughness was reassessed, as described for baseline analysis. Also, SMH was evaluated again by making one row of five indentations spaced at 100 μm parallel to the five baseline measurements, and the percentage of surface microhardness change (%SMHC) was calculated as follows: %SMHC = [(SMH after pH-cycling − SMH baseline)/SMH baseline] × 100.

Scanning electronic microscopy analysis

After the experimental procedures, three blocks were randomly selected from each G1 and G2 subgroup to assess the enamel surface topography by SEM. All these specimens and the three enamel blocks that had been set aside earlier for baseline surface topography were mounted on aluminium stubs, sputter-coated with gold, and examined in a scanning electronic microscope (JEOL-JSM, 5800LV; Tokyo, Japan), with an acceleration voltage of 15 kV. Scanning electron microscopy micrographs were taken at 30, 850, and 5000 magnification.

Statistical analysis

The data were analyzed using STATGRAPHICS 5.1 Software (Manugistics, Rockville, MD, USA). Initially, the normal distribution of the errors and the homogeneity of variances were checked, respectively, by Shapiro–Wilk's test and Levene's test. Based on these preliminary analyses, the surface Knoop microhardness was analyzed by two-way analysis of variance (ANOVA) and the roughness data were

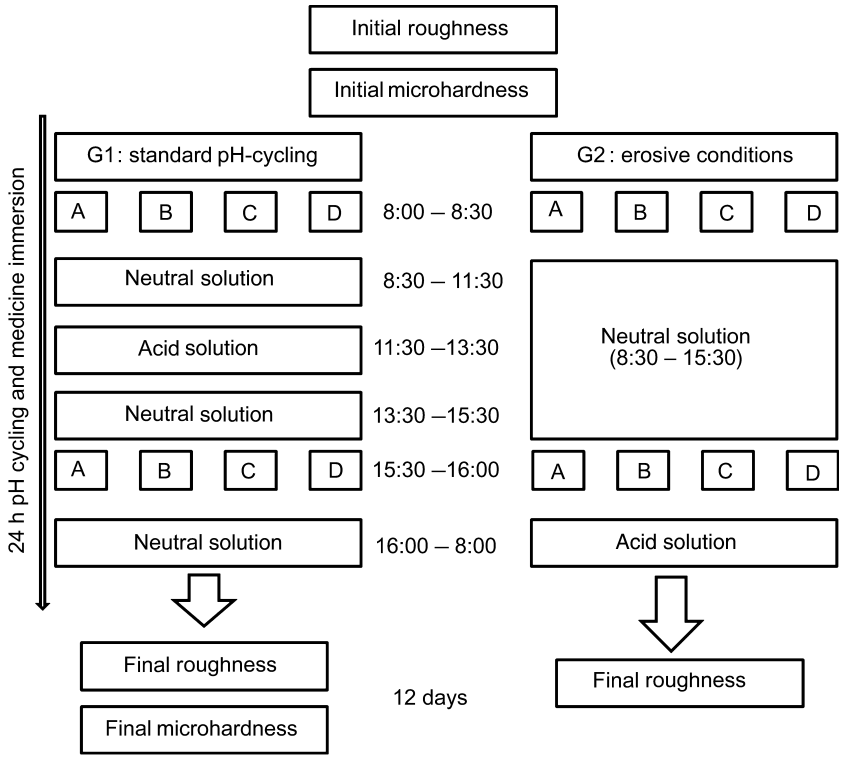

Fig. 1. Schematic design of the experimental protocol.

Table 2. Means and standard deviations of surface microhardness for G1 (simulation of physiological oral conditions without fluoride) before and after the treatments.

	Medicines/control			
pH-cycling	Klaricid®	Claritin®	Dimetapp Elixir®	Deionized water
Before	331.3 ± 27.7[a]	309.1 ± 26.5[a]	325.9 ± 27.0[a]	313.5 ± 19.0[a]
After	159.4 ± 10.6[b]	87.9 ± 11.5[c]	41.9 ± 16.0[d]	32.6 ± 8.8[d]

Values with same superscript letters are not statistically different. Two-way ANOVA followed by Tukey HSD test ($\alpha = 0.05$).

analyzed by multifactor analysis of variance. Tukey HSD multiple range test was applied to mean comparisons. The analyses were performed at a significance level of $\alpha = 0.05$. In addition, SEM images were analyzed qualitatively.

Results

Microhardness analysis

Table 2 summarizes the results of Knoop hardness for G1. All the specimens showed significant erosive patterns after treatment with the three medicines used and also the control (deionized water) ($P < 0.05$). The greatest reduction in microhardness was produced by deionized water (89.6%) and Dimetapp® (87.15%), followed by Claritin® (71.55%) and Klaricid® (51.9%); $P < 0.05$. Due to the extensive enamel destruction after the treatments in G2, it was not possible to verify the enamel microhardness of the specimens.

Roughness analysis

With regard to roughness, except for Claritin® in G1, all other medicines lead to a significant greater roughness ($P < 0.05$) after both pH-cycling regimens (G1 and G2) – Table 3. Multifactor ANOVA followed by Tukey HSD test also showed statistical significant differences

for roughness measurements between G1 and G2 for all treatments and control ($P < 0.05$).

In G1, Dimetapp® and deionized water gave the highest results for roughness with increases from 18.5% to 121.0%. For G2, the increase in roughness ranged from 1563.0% to 3389.5%. The results of Claritin®, Dimetapp® and deionized water were similar and were greater than Klaricid® ($P < 0.05$).

Scanning electron microscopy analysis

When compared to sound bovine enamel (Fig. 2), qualitative analysis of SEM images showed that all enamel specimens presented erosion patterns after both pH-cycling regimens (G1 and G2) and immersion to acidic medicines and deionized water (Figs 3 and 4). In G1, specimens exposed to Dimetapp Elixir® presented the most severely eroded areas, followed by those exposed to Claritin®, deionized water and Klaricid®. In G2, the morphological changes were much more pronounced than in G1, and followed the same sequence of severity – Dimetapp Elixir®, Claritin®, deionized water, and Klaricid®.

Discussion

It is well known that pH-cycling regimens are an efficient way to simulate the oral environment *in vitro*, submitting test specimens to the alterations with a pH that is commonly associated with this environment[18]. This *in vitro* study, however, aimed to verify whether liquid oral medicines could contribute to dental erosion not only in a physiological oral environment (G1) but also when a situation of high erosive challenge (G2), such as an excessive intake of acidic foods and drinks, is already established. Additionally, the option for performing a pH-cycling that simulated physiological oral conditions (G1) without fluoride increased its cariogenic and erosive potentials.

The medicines selected for this study presented characteristics that may increase their erosive potential (low endogenous pH and high titratable acidity) probably due to the

Table 3. Means and standard deviations of roughness for G1 (simulation of physiological oral conditions without fluoride) and G2 (erosive conditions) before and after the treatment.

	Medicines/control			
pH-cycling	Klaricid®	Claritin®	Dimetapp Elixir®	Deionized water
G1				
Before	$0.06 \pm 0.01^{A,a}$	$0.09 \pm 0.02^{A,a}$	$0.06 \pm 0.02^{A,a}$	$0.07 \pm 0.01^{A,a}$
After	$0.09 \pm 0.01^{A,b}$	$0.10 \pm 0.03^{A,a}$	$0.14 \pm 0.03^{A,b}$	$0.10 \pm 0.01^{A,b}$
G2				
Before	$0.07 \pm 0.01^{A,a}$	$0.05 \pm 0.01^{A,a}$	$0.06 \pm 0.01^{A,a}$	$0.06 \pm 0.01^{A,a}$
After	$1.18 \pm 0.74^{A,b}$	$1.98 \pm 0.73^{B,b}$	$1.97 \pm 0.48^{B,b}$	$1.54 \pm 0.45^{A,B,b}$

Within lines and groups (G1 and G2), values with the same superscript capital letters are not statistically different ($\alpha = 0.05$). Within columns and groups (G1 and G2), values with same lower case superscript letters are not statistically different ($\alpha = 0.05$). Multifactor ANOVA followed by Tukey HSD test ($\alpha = 0.05$).

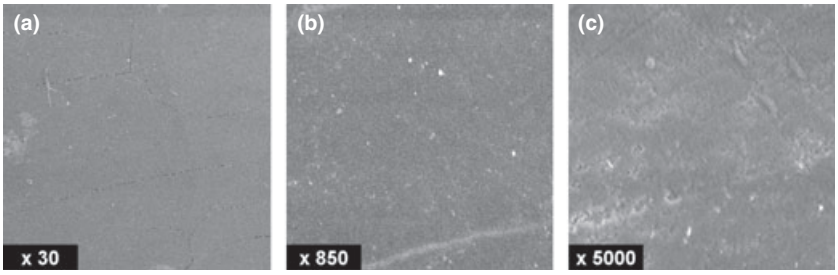

Fig. 2. Representative scanning electron microscopy photomicrographs of sound bovine enamel specimens. Photomicrographs are presented at original magnifications of ×30 (a), ×850 (b), and ×5000 (c).

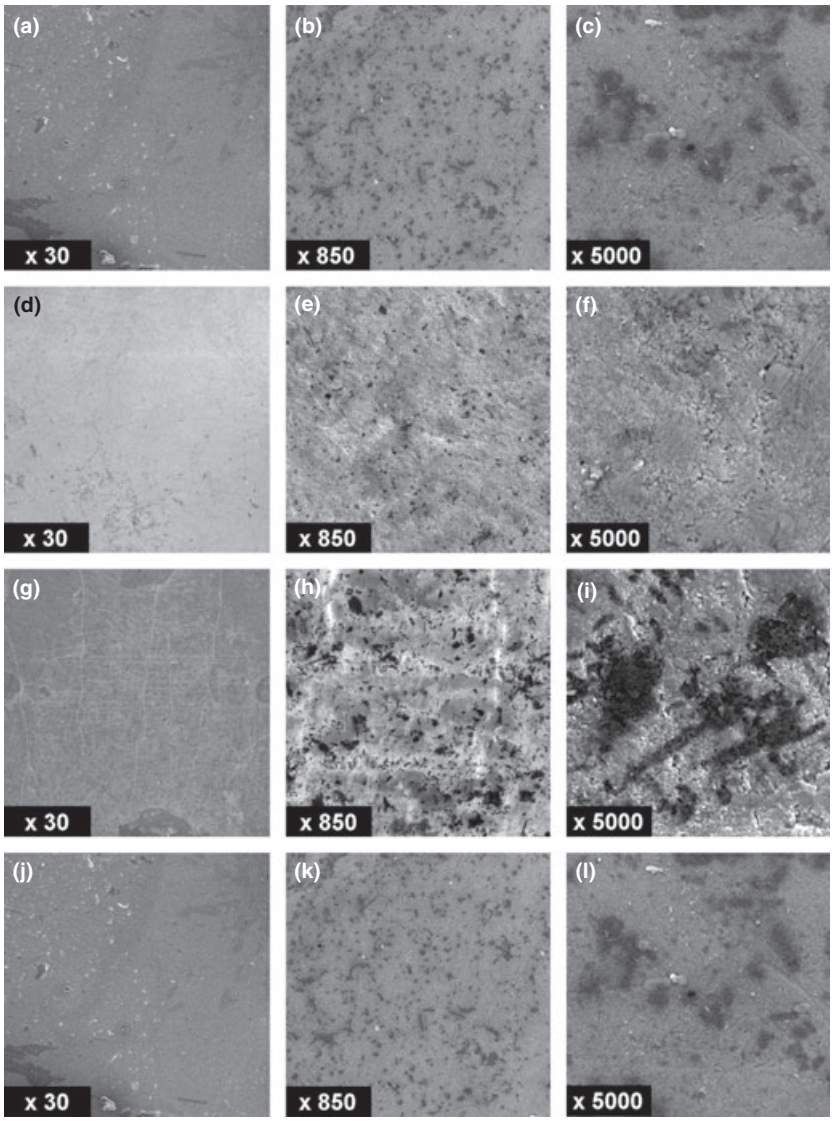

Fig. 3. Representative scanning electron microscopy photomicrographs of bovine enamel specimens exposed to pH-cycling model that simulated physiological oral conditions without fluoride (G1) and treated with Klaricid® (a, b and c), Claritin® (d, e and f), Dimetapp Elixir® (g, h and i) and deionized water – control (j, k and l). Photomicrographs are presented at original magnifications of ×30, ×850, and ×5000 from the left to the right for all treatments and control.

presence of citric acid[5]. The experimental conditions and exposure to medicines resulted in a substantial loss of mineral from the enamel blocks. The immersion of enamel in a neutral solution for 21 h daily, in Group 1, was not enough to prevent the demineralization by two 30-min immersions in the medicines. These findings are in accordance with the study of Costa *et al.* (2006)[5] that showed a significant decrease in SMH of primary enamel after pH-cycling and three 5-min immersions in an antihistamine-containing syrup and a nocturnal use of syrup, corresponding to an 8-h immersion. The nocturnal use of the antihistamine Claritin D® showed a significant surface microhardness change (SMHC), which was similar to the decrease in SMH that occurred in all subgroups of G1 (standard pH-cycling) in this study. In this study, the nocturnal use of medicines was not simulated. The twice daily 30-min exposure time was overestimated, however. This overestimation probably led to a more pronounced decrease in SMH in G1 when compared to the 5-min immersions performed in the study of Costa *et al.* (2006)[5].

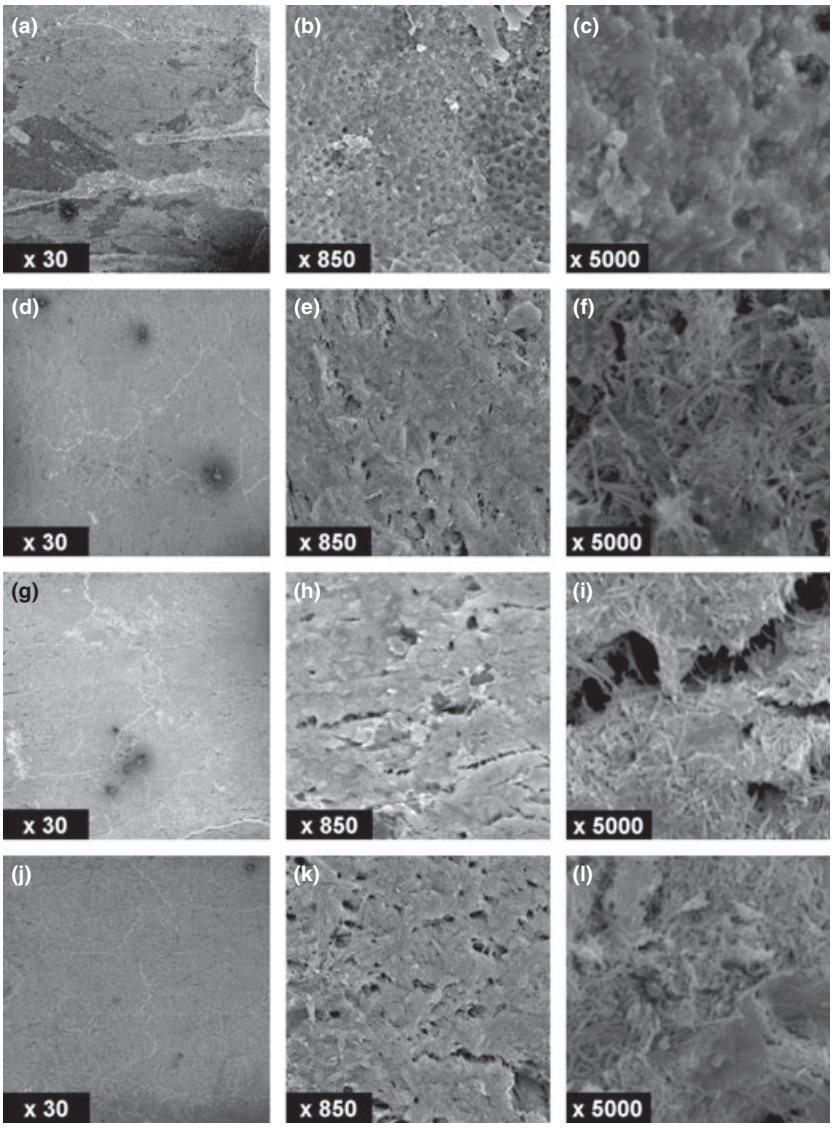

Fig. 4. Representative scanning electron microscopy photomicrographs of bovine enamel specimens exposed to an erosive pH-cycling model (G2) and treated with Klaricid® (a, b and c), Claritin® (d, e and f), Dimetapp Elixir® (g, h and i) and deionized water – control (j, k and l). Photomicrographs are presented at original magnifications of ×30, ×850, and ×5000 from the left to the right for all treatments and control.

Surface microhardness is the usual quantitative method employed to verify dental erosion along with SEM observations[19,20]. Roughness analysis has already been used to evaluate the effect of acidic medicines, under pH-cycling conditions, on the surface degradation of composite resins[13]. In this study, SMH proved to be limited in evaluating teeth submitted to a high erosive challenge because the indentations could not be carried out on the Group 2 specimens due to excessive surface enamel loss. Surface roughness showed alterations in both groups, but it was more pronounced in G2 where the roughness difference was about 20-fold greater than in G1.

Dental erosion promoted by both pH-cycling models (G1 and G2) may have been due to direct loss of the superficial enamel layer, which led to lower microhardness[20–23] for G1 and higher surface roughness for both G1 and G2. G1 (standard pH-cycling without fluoride) presented a lower variation (18.5–121.0%) in surface roughness when compared to G2 (highly erosive pH-cycling: 1563.0–3389.5%).

The large reduction in SMH in the G1 group, however, prompted us to speculate that an alteration of the innermost layer of enamel could be the initial sign of dental erosion. Therefore, an initial subsurface loss could be a sign for not only dental caries but also for dental erosion. This issue has already been described in a previous study, which demonstrated that an eroded lesion was associated with an area of slight subsurface mineral loss or softened enamel, which has been shown to be remineralizable[24]. The remineralization of eroded, etched and softened enamel by precipitation of various calcium phosphates has also been reported[25,26].

In this study, deionized water was chosen to be the immersion media of control groups because it would be inert to enamel and would not promote structural alterations; however, probably due to the acid attacks, Ca^{++} and PO_4^{--} ions from the enamel would have been released into the deionized water because of its unsaturated condition in respect to the enamel, promoting surface alterations and softening. This condition justifies the surface roughness and ultrastructural alterations viewed by SEM, and the enamel softening, marked by low values of SMH for the control subgroup in G1.

The erosive effect of dietary acids on dental tissue can be influenced by a number of factors, including pH, pKa, titratable acidity, temperature, acid character, concentration, and chelation potential. Furthermore, frequency, timing of intake, time in the mouth, fluoride content, pellicle layer and variations in tooth structures are thought to be of importance and are understood to be an integral part of dietary acid tooth erosion[27]. An increase of pH, often accompanied by the addition of calcium and/or phosphate salts, has been shown to reduce the erosive potential of soft drinks *in situ* and *in vitro*[28–31]. Many studies have found the erosive potential of drinks to be associated with their low calcium and phosphate concentrations[2,29,31,32]. The addition of relatively small amounts of calcium to citric acid solutions was found to reduce the loss of enamel and this effect was observed progressively as the pH was increased[33]. Probably, the higher pH and the greater amount of calcium, fluoride and phosphate in Klaricid® compared to the other medicines tested, promoted the lowest erosion pattern observed in both subgroups (G1 A and G2 A).

It is also important to note that the medicines evaluated had high viscosity values, the highest being Klaricid®. In clinical practice, viscous drinks are likely to adhere to teeth and inner mouth surface and will, therefore, be held in the mouth for a longer period of time[34]. This would allow possibly penetration into fissures and proximal areas of teeth that are inaccessible to the toothbrush. Consequently, high viscosity medicines could increase their harmful effects. Although the specimens in this study were rinsed with deionized water after immersion in the medicines, it is possible that Klaricid®, the most viscous medicine, may have been retained on the enamel surface as a kind of pellicle, which may have provided a protective effect against acid attacks of the acid solution. This hypothesis could also account for the lowest surface changes with Klaricid.

The difficulty to reproduce a clinical situation in *in vitro* studies must be kept in mind. This is due to the complexity of the oral environment. The absence of buffering by saliva and of salivary pellicle for example, which leads to the media having direct contact with the teeth, is an aggravating factor[35,36]. Nevertheless, some situations may also occur in the oral cavity, such as the chronic use of medicines that besides being highly acidic, can also reduce salivary flow, like the antihistamines (Claritiitin® and Dimetapp Elixir®) tested here. The label on Dimetapp Elixir® even recommends its use up to six times/day, which increases the chances of an erosive challenge.

In this physicochemical model of remineralization and demineralization, the presence of microorganisms and medicine sugar concentrations were not taken into consideration, but these factors could play a role in the effects of these medicines *in vivo*. From a clinical point of view, it could be hypothesized that these acidic medicines would, probably, lead to a drop in dental biofilm pH, increasing its acidogenicity. Some studies have already

reported that medicines with high concentrations of sucrose and low endogenous pH have both cariogenic and erosive potentials[5,12], since they promote a rapid drop in oral pH, which remains low for long periods of time.

Another interesting point is that some studies reported that acid affects bovine enamel more than human enamel[37,38]. In addition, removal of the outermost layer of enamel during specimen preparation is thought to influence the severity of the erosion process[39]. Therefore, the results presented here may be overestimated when compared to an *in vivo* process. Alternatively, *in situ* studies to evaluate the effect of acidic medicines on human enamel could determine the real extension of the problem.

From the experimental conditions adopted in this study, it could be concluded that the three acidic medicines and the pH-cycling models promoted enamel erosion, which was more pronounced in the subgroups in which Dimetapp® (medicine with the lowest pH and lowest viscosity) and deionized water were used. Furthermore, Klaricid® (medicine with the highest pH and highest viscosity) seemed to present an *in vitro* protective effect against acid attacks which could be explained by its mineral content as well as its viscosity.

What this paper adds

- This paper provides a good basis for a more complete study looking at some of the variables mentioned in the discussion.

Why this paper is important for paediatric dentists

- Knowledge of the properties of oral liquid medicines that damage dental enamel assists the pharmaceutical industry to introduce medicines with new formulations that reduce the potential risk for dental caries and dental erosion.

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