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ORIGINAL ARTICLE

In vitro effect of paediatric liquid medicines on deciduous enamel exposed to biofilm

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Abstract

Objective. This study aimed to assess the *in vitro* effects of paediatric liquid medicines on deciduous enamel exposed to biofilms. ***Methods.*** Fragments ($n = 25$) of first primary molars were covered by nail varnish, leaving a 22 mm^2 exposure area. Specimens were fixed in polystyrene plates containing BHI broth media. Pooled human saliva was added to form a mature biofilm on fragments over a 10-day period in microaerophilic conditions. Specimens were divided into groups ($n = 5$ per group) and treated (50 μL) daily for 1 min over 1 week as follows: G1 = 10% sucrose solution (positive control); G2 = Dimetapp Elixir® (antihistamine); G3 = Claritin® (antihistamine); and G4 = Klaricid® (antibiotic). Five other fragments, without treatment and inoculum represented the blank controls. The covered area for each specimen represented the negative control. Cross-sectional hardness of the enamel was used as a demineralization indicator. ***Results.*** All treatment groups showed hardness loss compared to the corresponding negative controls ($p < 0.05$). Among the treatment groups, G2 exhibited the greatest demineralization pattern ($p < 0.05$) followed by G3, G1 and G4. ***Conclusion.*** All medicines caused deciduous enamel demineralization in the presence of biofilm. The greatest hardness loss was observed after treatment with Dimetapp Elixir®.

Key Words: *dental biofilm, microhardness, deciduous tooth, use of medications*

Introduction

Treatments for the paediatric population commonly employ syrups and oral suspensions [1]. Some parents associate susceptibility to dental caries to frequent use of these liquid medicines by their children at early ages [2]. According to Feigal et al. [3], some factors, such as the addition of sugar (sucrose, fructose and glucose) to paediatric liquid medicines, may lead to this cause–effect relationship.

Sugar is used to mask the flavour of these medicines and, thus, to increase compliance with treatment [2–4]. However, sugar is metabolized by bacteria from biofilm; constant contact with teeth favours the appearance of dental caries because it causes a rapid decrease of oral pH [5,6]. Moreover, paediatric liquid medicines often have low pH values, low ionic concentrations (calcium, phosphate and fluoride) and high viscosities, which increase their cariogenic potentials [5,7,8].

From the theoretical point of view, these medicines have cariogenic and erosive potential; however, there is still no evidence of this relationship in the literature, especially when dental biofilm is present. Previous *in vitro* studies analysing the effects of some medicines on dental enamel have revealed methodological limitations precisely because they did not include biofilm in their experimental models but instead focused only on the physicochemical features of enamel dissolution [1,8–11].

Fontana et al. [12] stated that a bacterial system for biofilm formation is the best *in vitro* model to develop caries lesions. Therefore, this study aimed to

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Table I. Characteristics of the liquid medicines.

Characteristics	Klaricid® (antibiotic)	Claritin® (antihistamine)	Dimetapp elixir® (antihistamine)
Concentration of sugar (g%)	77.46 (sucrose)	86.90 (glucose + fructose)	75.93 (glucose + fructose)
pH	5.04	2.80	2.70
Viscosity at 20 s^{-1} (cP)	1660	19.70	13.30

According to Valinoti et al. [14] and Neves et al. [13].

assess *in vitro* cross-sectional alterations in hardness of enamel exposed to paediatric syrups (antihistamines) and oral suspension (antibiotic) in the presence of a mature biofilm as a demineralization indicator.

Materials and methods

Medicines

Paediatric liquid medicines in the form of syrups and oral suspensions were used in this study. Claritin® (antihistamine; Schering-Plough, Vila Olímpia, Brazil) and Dimetapp Elixir® (antihistamine; Wyeth, São Paulo, Brazil) represented syrups and Klaricid® 50 mg/mL (antibiotic; Abbott, São Paulo, Brazil) represented oral suspension. Syrups were chosen on the grounds of a previous study [13] that identified them as having the highest sugar concentrations, lowest pH values and high viscosities among a group of eight investigated syrups. Regarding the chosen oral suspension, Valinoti et al. [14] showed that it exhibited one of the highest sugar concentrations among 29 antibiotics commonly taken by children, along with a lower pH value and a high viscosity (Table I).

Analysis of calcium, fluoride and phosphate concentrations in the chosen medicines

The concentration of calcium was established by atomic absorption spectrophotometry. Fluoride and phosphate concentrations were assessed by fluoride ion selective electrode [15] and colourimetric assays [16], respectively.

Sample selection and preparation

A convenience sample of 15 healthy deciduous first molars were obtained; teeth were voluntarily donated by patients at the Paediatric Dental Clinic of the Federal University of Rio de Janeiro according to the local Ethics Committee regulations. Teeth were kept in 2% formaldehyde solution (pH 7.0) for disinfection purposes. Elements had been previously selected after observation under a stereoscopic microscope (Zeiss; 475200 9901, Oberkochen, Germany) at 40× magnification. Elements exhibiting structural alterations ($n = 2$) were excluded from the study. After selection, teeth (n = 13) were sectioned along the mesiodistal axis, resulting in two fragments (Figure 1). From these, 25 fragments were covered with nail varnish, leaving a 22 mm^2 exposure area (Figure 1). The covered area represented the negative control in this study because it was not exposed to treatment. All specimens were sterilized with ethylene oxide (Bioxxi, Sterilization Services, Rio de Janeiro, Brazil) before being subjected to the experimental protocol.

Inoculum used for biofilm formation on fragments

The inoculum used was composed of non-stimulated human saliva collected from three volunteers (one male and two females) with good general and oral health complying with the Zero [17] inclusion and exclusion criteria, as follows: none of the subjects were on any medication; they did not have any caries activity; and they did not use orthodontics devices. Also, the subjects gave their informed consent to be included in the present study.

Subjects were instructed not to consume food or beverages except water for 1 h before saliva collection and not to brush their teeth for 24 h before the experiment (saliva collection). One millilitre of saliva from each volunteer was placed into a single test tube that was then agitated on a vortex, thus forming a human saliva 'pool' containing 2×10^8 CFU/mL (1:200 dilution). A total of 0.6×10^3 CFU/ml of *Streptococcus mutans* were identified in this suspension.

In vitro formation of a mature biofilm on dental blocks and treatment of biofilm

The biofilm formation model suggested by Antonio et al. [18] was used here; each specimen was randomly fixed inside one well of a 24-wellpolystyrene plate (TPP, Zellkultur Testplatte 24 F) by means of 400 µl of 2% agar (Noble Agar, Difco, Sparks, USA). Culture medium (BHI, Difco, 1485 µL/well) and inoculum (15 µL) were added to each well. The system was incubated under microaerophilic conditions for 10 days at 37°C and a mature biofilm developed over these enamel blocks (Figure 1). According to the literature [19], a mature biofilm is characterized by the attachment of new microorganisms on the microbial community either by recruitment from planktonic bacteria or through replication of cells already present in the biofilm.

After biofilm formed on fragments, specimens were treated once daily according to the following groups ($n = 5$): G1 = 10% sucrose solution (positive control); G2 = Dimetapp Elixir® (antihistamine); G3 = Claritin® (antihistamine) and G4 = Klaricid® (antibiotic). In addition, another five fragments without inoculum did not receive any treatment, thus representing the blank control. The surfaces of the specimens received 50 µL of each tested substance, which was left on the

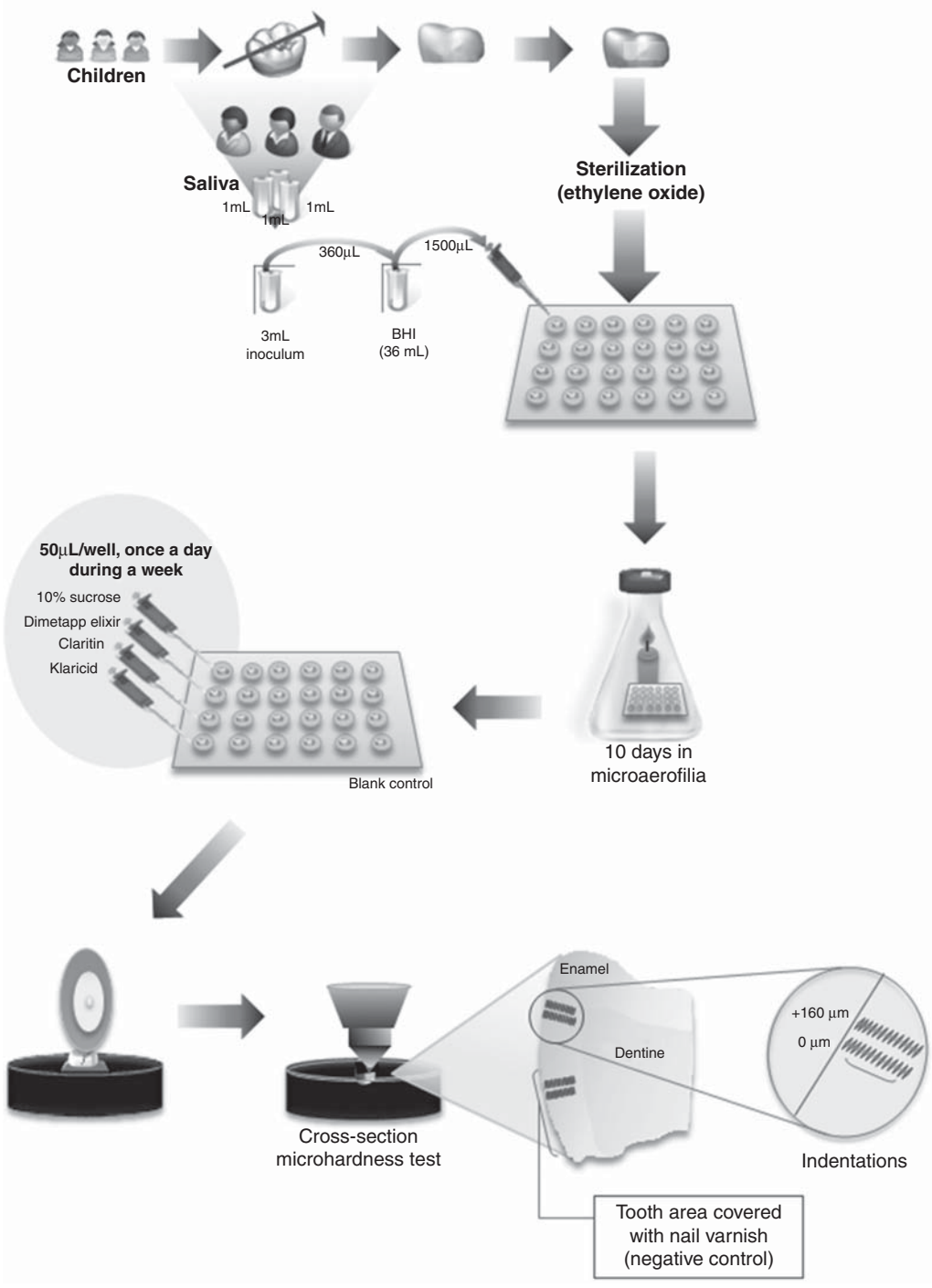

Figure 1. The biofilm model and the microhardness test used to identify *in vitro* cross-sectional alterations in hardness of enamel exposed to paediatric syrups (antihistamines) and oral suspension (antibiotic).

fragments for 1 min. The fragments were then cleansed twice with 1500 μL of deionized water. After cleansing, fresh culture medium (BHI = 1500 μL) without inoculum was added to each well. The plate/specimens/biofilm system was again incubated under microaerophilic conditions until the following day, when treatment was performed again. Treatment was thus performed for 7 consecutive days, always at the same time of day (Figure 1). The viability of the inoculum was confirmed in each treatment day through the medium density.

Cross-sectional hardness assessment

After 7 days of treatment, the blocks were removed from the plate, longitudinally sectioned (Figure 1) and polished under refrigeration [20] to perform the blind cross-sectional hardness assessment test. The

Table II. Ionic content of the liquid medicines.

Ionic content (μg/ml)	Calcium	Phosphate	Fluoride
Klaricid® (Clarithromycin)	11.16	33.32	0.17
Claritin® (Loratadine)	10.98	< 1.5	< 0.025
Dimetapp elixir® (Pseudoephedrine + brompheniramine)	9.82	< 1.5	< 0.025

test was performed using a micro-durometer (Buehler, Lake Bluff, USA) with a Knoop-type diamond penetrator under a load of 25 g charge for 10 s. Two 14-indentation columns separated 150 μm from each other were made at the following depths: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 200 and 300 μm, using the enamel surface as a reference (Figure 1). Indentations were made in the areas exposed to treatment and in the control area (the area covered with nail polish, which represented the negative control). Measurements were made for each depth level and the results were expressed as Knoop hardness [18].

Statistical analysis

The data were analysed using the statistical software SPSS version 17.0 (SPSS Inc., Chicago, IL). The Shapiro-Wilk test allowed the establishment that the distribution of results was not normal. Because each specimen had its own (negative) control, a non-parametric Wilcoxon test was used in the following paired analyses: treatment vs negative control and blank control vs negative control. A Kruskal-Wallis test was used to investigate the statistical differences among groups, and a Mann-Whitney test was used to compare pairs of groups. The level of significance was 5% in all tests.

Results

The ionic characteristics of the liquid medicines are described in Table II. All treatment groups exhibited higher mineral loss compared to their respective controls (Wilcoxon, $p < 0.05$) (Figure 2). The blank control group and its corresponding control were not significantly different (Wilcoxon, $p > 0.05$) (Figure 3). Among the treatment groups, G2 (Dimetapp Elixir® antihistamine) exhibited the greatest demineralization pattern, followed by groups G3 (Claritin® antihistamine), G1 (10% sucrose solution) and G4 (Klaricid® antibiotic) (Mann-Whitney, $p < 0.05$) (Figure 4).

Discussion

This study employed paediatric liquid medicines with high sugar concentrations, high viscosities and low pH values. These factors increase the cariogenic potential

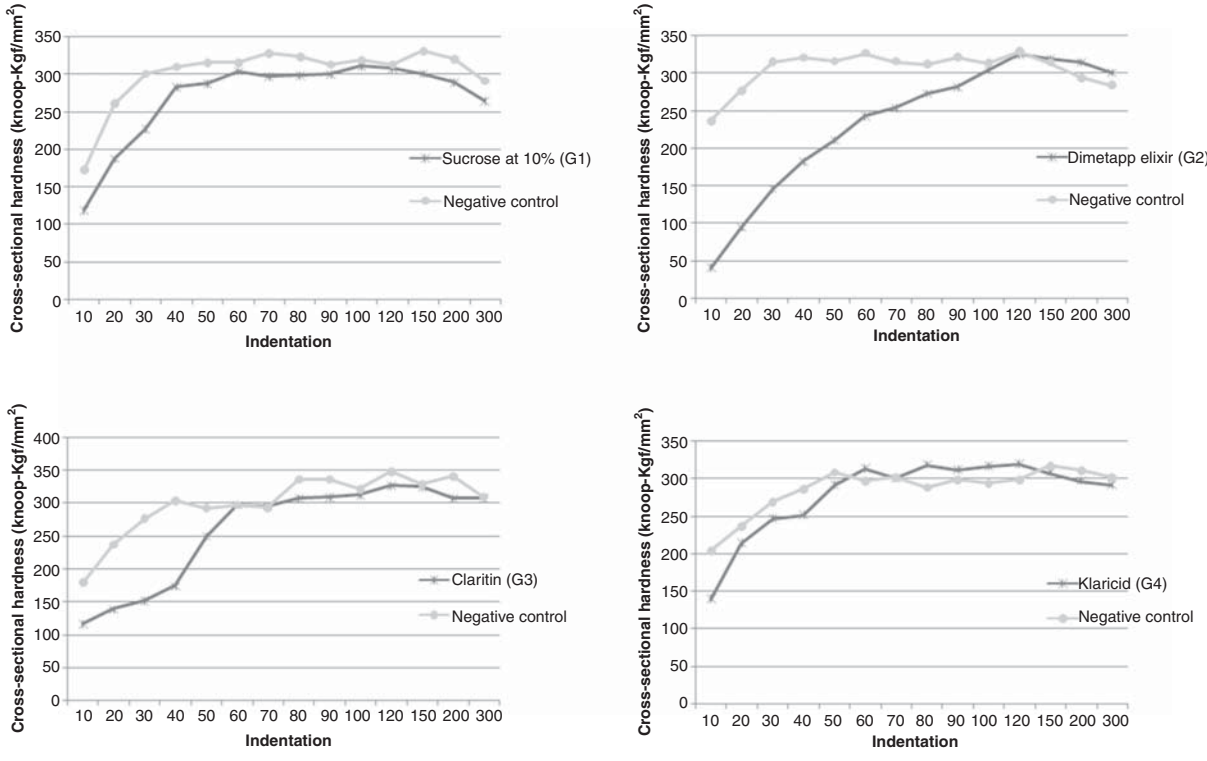

Figure 2. Means of enamel Knoop hardness (Kgf/mm^2) after treatments with G1 (10% sucrose solution; positive control), G2 (Dimetapp Elixir®; antihistamine), G3 (Claritin®; antihistamine) and G4 (Klaricid®; antibiotic) compared to their respective controls. All groups showed a loss of hardness when compared to their respective controls (Wilcoxon, $p < 0.001$).

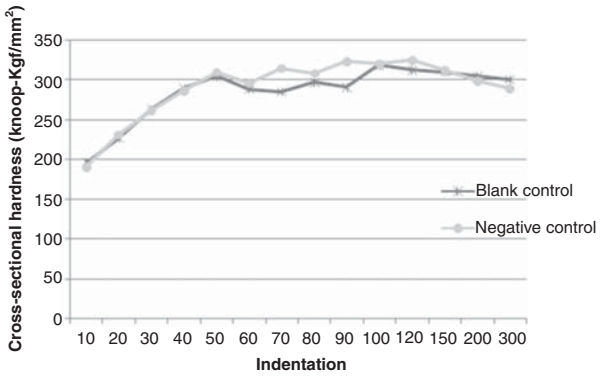

Figure 3. Means of enamel Knoop hardness (Kgf/mm^2) of blank control group compared to its respective control (negative control). No significance difference was observed among blank control and negative control (Wilcoxon, $p > 0.05$).

of such medicines [7], therefore, to assess the effects of these substances on deciduous enamel is perfectly justified because paediatric liquid medicines are commonly used with children.

The *in vitro* biofilm formation model suggested by Antonio et al. [18] was chosen because it best reproduces the oral conditions for *in vitro* studies, since it uses bacteria as well as deciduous teeth, properly to mimic an oral environment. According to Fontana et al. [12], an *in vitro* study model including a bacterial biofilm system best represents the cariogenic challenges occurring in the oral cavity. This model was employed in this study to simulate the behaviour of the investigated medicines when in contact with the biofilm accumulating over the teeth of paediatric patients, thus assessing whether they favour caries formation as a function of the sugar in their compositions.

The formation of caries is characterized by sub-surface mineral loss in the enamel. Therefore, the cross-sectional microhardness test used in this study was relevant, as it assessed enamel hardness from the superficial layer towards the dentin, thus allowing the identification of deciduous enamel mineral loss or gain after different treatments. Moreover, Featherstone et al. [21] stated in 1983 that the cross-sectional microhardness test is an excellent method for caries assessment because there is an excellent correlation between enamel microhardness and lesion mineral percentage.

Still considering the methods, the sample size was a limitation of this study. The authors used deciduous teeth as experimental samples. Teeth are more difficult to be obtained than hydroxyapatite discs or glass slides, which are devices commonly used in biofilm models. Actually, we opted for teeth because they mimic what occurs in the oral cavity more accurately. In addition, the sample size problem was minimized through the use of a paired sample (all groups were paired with its own teeth—the covered area). Moreover, considering the results of each group, there was not a great difference among the standard deviation values, when they were individually analysed.

Microhardness results indicated that all of the medicines promoted hardness loss compared to the negative control. This finding may be explained by the high sugar concentrations and viscosities and the low pH values of the investigated medicines. Moreover, the negative control was not exposed to either medicines or bacteria-containing culture medium because it was covered by nail polish. To our knowledge, the cariogenic potential of these medicines had not been tested through this bacterial model until the present study.

According to the literature [22–24], when biofilm remains adhered to the teeth for more than 3 days, the pH decreases in the biofilm, leading to mineral loss. When minerals are not reincorporated by means of re-mineralization with increasing pH, dental caries become unavoidable. The finding that antihistamine in syrup form (Dimetapp Elixir®) exhibited the greatest demineralization pattern compared to the remainder of the tested medicines was probably due to its lower pH and lower calcium, phosphate and fluoride concentrations in addition to its high sugar concentration.

The 10% sucrose solution caused greater enamel demineralization compared to both blank and negative controls, but was not different compared to the medicines. Although 5% sucrose solution is known to form cariogenic biofilm [25], when it is associated with a low ionic concentration and a low pH, as present in the formulae of the medicines, caries formation might be potentiated.

Among the three medicine groups, the antibiotic Klaricid® exhibited the lowest amount of demineralization. Some studies show that dental biofilm formed in the presence of sucrose is more cariogenic

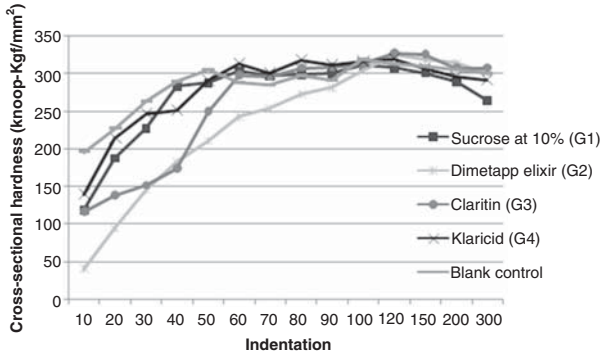

Figure 4. Means of enamel Knoop hardness (kgf/mm^2) for each group. The antihistamine Dimetapp Elixir® had the highest demineralization pattern when compared to all other groups (Mann Whitney, $p < 0.001$). G2 exhibited the greatest demineralization pattern ($p < 0.001$) followed by G3, G1 and G4.

compared to biofilm formed in the presence of glucose and fructose [15]. Although Klaricid® contains sucrose in its composition, it also possesses antibacterial properties, which possibly resulted in decreased acid production by plaque micro-organisms and, consequently, it provoked less mineral loss in the tested fragments. Moreover, this medicine contains the highest fluoride concentration, which might have favourably affected dental re-mineralization in the corresponding fragments. Thus, studies involving antibiotics without sugar addition and with and without fluoride in their compositions become relevant.

There was no significant difference between the blank and the negative controls. Thus, it was verified that the BHI culture medium compounds did not affect the dental enamel hardness. Furthermore, regarding sucrose treatment group (G1), the fragments exhibited lower hardness values compared to controls, which means this study model can be accurately replicated.

Conclusions

On the grounds of these findings, we may conclude that all tested medicines promoted enamel demineralization in the presence of a mature biofilm. Therefore, whereas caries disease is likely preventable, the results of this study are a warning to paediatricians and caretakers that there are risks involved in the continuous use of the investigated paediatric liquid medicines. It is worthwhile to emphasize, however, that, because this was an *in vitro* study, further studies are needed to verify the cariogenic effect of these medicines on dental enamel.

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***Declaration of interest*:** The authors report no conflicts of interest.

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