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Research paper

The effect of magnesium hydroxide-containing dentifrice using an extrinsic and intrinsic erosion cycling model

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ABSTRACT

Objective: To evaluate, *in vitro*, the effect of $Mg(OH)_2$ dentifrice, and the influence of the number of experimental days, on the extrinsic (citric acid –CA) and intrinsic (hydrochloric acid –HCl) enamel erosion models.
Design: Human enamel slabs were selected according to surface hardness and randomly assigned to 3 groups (n = 9) as follows: non-fluoridated (negative control), NaF (1450 ppm F- positive control) and $Mg(OH)_2$ (2%) dentifrices. The slabs were daily submitted to a 2-h period of pellicle formation and, over a period of 5 days, submitted to cycles (3×/day) of erosive challenge (CA 0.05 M, pH = 3.75 or HCl 0.01 M, pH = 2 for 30 s), treatment (1 min −1:3 w/w of dentifrice/distilled water) and remineralization (artificial saliva/120 min). Enamel changes were determined by surface hardness loss (SHL) for each day and mechanical profilometry analysis. Data were analyzed by two-way ANOVA followed by Tukey's test to % SHL and one-way ANOVA to profilometry ($p < 0.05$).
Results: The number of experimental days influenced the erosion process for the two types of erosion models ($p < 0.001$). $Mg(OH)_2$-containing dentifrices were effective in reducing enamel extrinsic acid erosion as determined by % SHL ($p < 0.001$) when compared to the control group, being better than positive control ($p < 0.001$); however, the dentifrices were not effective for the intrinsic model ($p = 0.295$). With regards to surface wear, no statistically significant differences were found among the groups for CA ($p = 0.225$) and HCl ($p = 0.526$).
Conclusion: The findings suggest that $Mg(OH)_2$ dentifrices might protect enamel against slight erosion, but protection was not effective for stronger acid erosion.

1. Introduction

The frequent ingestion of citrus fruits, acidic juices, carbonated drinks or sports drinks, as well as gastrointestinal disorders that cause the presence of gastric acid in the oral cavity may lead to loss of dental hard tissues (Lussi, Schlueter, Rakhmatullina, & Ganss, 2011; Johansson, Omar, Carlsson, & Johansson, 2012). According to Duffey et al. (2012) adolescents consume an average of 360 ml of acid drinks per day, which indicates a high level of ingestion of acid-containing products. Furthermore, a systematic literature review demonstrated a median prevalence of 24% for tooth erosion in adult patients with gastro-esophageal reflux disease (Pace, Pallotta, Tonini, Vakil, & Bianchi Porro, 2008), proving that gastric acid is also an important etiological factor for dental erosion.

Avoiding a lifelong contact of erosive acidic contents with dental surfaces is an impossible task. Therefore, the development of early diagnostic methods and adequate preventive measures should be researched (Lussi et al., 2011). Many preventive measures are based on the release of fluoride compounds by oral solutions or dentifrices through daily use and easy access to over the counter products (Ganss, Lussi, Grunau, Klimek, & Schlueter, 2011; Passos, de Vasconcellos, Pequeno, Rodrigues, & Santiago, 2015; Scaramucci, Borges, Lippert, Frank, & Hara, 2013). The protective effect of ionic fluoride from NaF (sodium fluoride) dentifrices on enamel erosion has been showed in some researches, once it forms CaF_2-like products, which can protect this tissue against acid challenges (Ganss et al., 2011; Passos, Santiago, Tenuta, & Cury, 2010; Passos et al., 2015; Soares et al., 2017).

However, allergic patients to fluoride products (de Groot, Tupker, Hissink, & Woutersen, 2017; Van Baelen, Kerre, & Goossens 2016) require other strategies for prevention of dental erosion. Recently, Abdallah et al. (2016) showed that magnesium ions react with enamel during dissolution and precipitation process improving the physical properties of enamel – hardness increase. To the best of the current authors' knowledge, there is no published data on the effects of dentifrices containing magnesium ions on enamel surfaces exposed to simulated exogenous or endogenous acids.

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Different neutralizing products have been assessed to reduce erosion (Lindquist, Lingström, Fändriks, & Birkhed, 2011; Messias, Serra, & Turssi, 2008; Messias, Turssi, Hara, & Serra, 2010; Turssi et al., 2012). The protective effect of antacid products on dental erosion has been shown in some studies (Messias et al., 2010; Turssi et al., 2012). According to Lindquist et al. (2011) these products increase the intra-oral pH after an erosive process, presenting a buffering effect (Meurman, Kuittinen, Kangas, & Tuisku, 1988). Furthermore, this effect might be caused by these products reacting with the acid and forming a salt (Turssi et al., 2012). Messias et al. (2010) indicated that oral rinsing containing sodium bicarbonate helped to minimize the destructive effect caused by erosive challenges.

The aim of the present study was to assess the effect of magnesium hydroxide, fluoride- and non-fluoride-based dentifrices on reducing the progression of enamel surface erosion originated by extrinsic acid (experiment one) or intrinsic acid (experiment two). In addition, the influence of the number of experimental days on enamel surface softening was evaluated. The null hypothesis tested was that there is no difference among the tested dentifrices against both kinds of acid challenges.

2. Material and methods

2.1. Preparation of enamel samples

The study protocol was reviewed and approved by the local Research and Ethics Committee (protocol #75/12). Enamel slabs were obtained from caries-free human third molars that had been stored in 0.01% (w/v) thymol solution at 4 °C (Passos et al., 2015). Enamel slabs (4 × 4 × 2 mm) were cut from the middle third of the coronal surface. Each slab was sequentially ground using a water-cooled mechanical grinder (Ecomet/Automet 250 Grinder-Polisher; Buehler Ltd, Lake Bluff, IL, USA) with 400-, 600-, and 1200-grit Al_2O_3 papers and polished using cloths with a 1 μm diamond suspension – Alpha Micropolish; Buehler Ltd, Lake Bluff, IL, USA).

A total of fifty-four enamel slabs were randomly divided into experimental groups based upon their baseline surface hardness values (SHbas), using a computer generated list (Microsoft Excel 2007). The SHbas values were determined by placing five indentations, 100 μm apart from each other, at the center of the specimens using a Knoop indenter with a load of 50 g and a dwell time of five seconds (FM100, Future Tech, Tokyo, Japan). Enamel specimens presenting a mean hardness of 328.1 ± 13.1 kg/mm^2 were selected and allocated to three groups for experiment one and experiment two (n = 9), generating balanced groups.

Subsequently, two parts of each specimen were covered with a dark-colored acid-resistant varnish (Jordana Cosmetics Corp., Los Angeles, CA, USA) to serve as the reference area for profilometry analysis. The exposed area of 2 × 4 mm in the center of each specimen was subjected to the treatments. In experiment 1, the acid challenge was performed using 0.05 M citric acid (citric acid dehydrated, pH 3.75; Dinâmica®, Diadema, SP, Brazil), while in experiment 2, 0.01 M hydrochloric acid (pH 2.0; Merck, Darmstadt, Germany) was used. The experimental groups were: non-fluoridated (negative control, pH = 6.86; basic ingredients: sorbitol, sodium lauryl sulfate, sodium hydroxide, hydrated silica), NaF (1450 ppm F; positive control; pH = 7.36; basic ingredients: sorbitol, sodium lauryl sulfate, copolymer, sodium hydroxide, sodium fluoride, triclosan, hydrated silica) and $Mg(OH)_2$ (0 ppm F; 2%; pH = 9.96; basic ingredients: calcium carbonate, magnesium hydroxide, sodium lauryl sulfate, magnesium sulfate) dentifrices.

2.2. Pellicle formation

Fresh saliva samples were collected each day from groups of 15–20 volunteers without active carious lesions, erosions, or salivary dysfunction. The subjects did not eat or smoke for the 8-h period before sampling. Saliva was stimulated with paraffin wax for five min. Saliva from the first minute of chewing was swallowed, and the rest was collected and deposited into 50-ml centrifuge tubules. The saliva samples were centrifuged for 10 min at 2000 rpm in a pre-cooled centrifuge (4 °C) (*5415R*, Eppendorf, Brazil) (de-Melo et al., 2011). The clear fluid above the sediments was pooled and used for pellicle formation (Nekrashevych & Stösser, 2003). Each group of enamel slabs was independently immersed in the clarified saliva and incubated for a period of two hours each day before the erosive challenges, under agitation at 100 rpm (5 ml per slab) and 37 °C to simulate the oral cavity temperature.

2.3. Experimental procedures

The study consisted of two separate experiments. Both experiments were cyclic procedures, repeated over a five–day period, and included pellicle formation, erosion, treatments with the dentifrices and remineralization using artificial saliva (1.5 mM Ca; 0.9 mM PO_4; 150 mM KCl and 0.1 M Tris buffer, pH 7.0–5 ml per specimen) (Queiroz, Hara, Paes Leme, & Cury, 2008).

For each experimental day, all procedures were performed under agitation at 100 rpm and at 37 °C. All specimens were immersed in clarified saliva for 2 h prior to experimentation to allow for the formation of pellicle. Subsequently, each slab was submitted to a citric acid or hydrochloric acid solution (5 ml per specimen) for 30 s. The specimens were then treated with fresh dentifrice slurry (5 ml per specimen) for one minute that had been prepared from non-fluoridated, magnesium hydroxide or sodium fluoride dentifrices (1 part toothpaste to 3 parts distilled water solution, by weight). The slurries were freshly prepared at the beginning of each experimental day. Next, each slab was rinsed with distilled water and immersed in artificial saliva for two hours. This cycle was repeated three times a day for five days. At the end of each experimental day, the slabs were evaluated for surface hardness (Table 1).

2.4. Measurement of enamel surface loss

Measurements of enamel surface loss were performed using the stylus profilometer, Hommel Tester T1000 (Hommelwerke GmbH, Germany) after the last experimental day. The difference between the heights (H) of the surfaces of the reference and the treated areas was evaluated. Before the analysis, the nail varnish was carefully removed, exposing the untreated reference areas. On each sample, at intervals of 100 μm, five profile traces (1.5 mm in length) were recorded, the levels of enamel wear, in micrometer (μm), were determined in relation to the reference surfaces. For each sample, the mean values obtained from the five traces were calculated.

Table 1
Daily cycling.

Steps	Sequence	Treatment
1	Pellicle formation	Human saliva (2 h)
2	Erosive challenge	Citric acid (30 s) – Experiment 1 Hydrochloric acid (30 s) – Experiment 2
3	Treatment with dentifrice/destilled water	Non-fluoride (1 min) Magnesium Hydroxide (1 min) Sodium Fluoride (1 min)
4	Remineralization Repeat step 2 Repeat step 3 Repeat step 4 Repeat step 2 Repeat step 3 Repeat step 4	Artificial saliva (2 h)
5	Remineralization	Artificial saliva (overnight)
All steps in 37 °C and agitation		