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Effect of Beverages on Bovine Dental Enamel Subjected to Erosive Challenge with Hydrochloric Acid

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This study evaluated by an *in vitro* model the effect of beverages on dental enamel previously subjected to erosive challenge with hydrochloric acid. The factor under study was the type of beverage, in five levels: Sprite® Zero Low-calorie Soda Lime (positive control), Parmalat® ultra high temperature (UHT) milk, Ades® Original soymilk, Leão® Ice Tea Zero ready-to-drink low-calorie peach-flavored black tea and Prata® natural mineral water (negative control). Seventy-five bovine enamel specimens were distributed among the five types of beverages (n=15), according to a randomized complete block design. For the formation of erosive wear lesions, the specimens were immersed in 10 mL aqueous solution of hydrochloric acid 0.01 M for 2 min. Subsequently, the specimens were immersed in 20 mL of the beverages for 1 min, twice daily for 2 days at room temperature. In between, the specimens were kept in 20 mL of artificial saliva at 37°C. The response variable was the quantitative enamel microhardness. ANOVA and Tukey's test showed highly significant differences (p<0.00001) in the enamel exposed to hydrochloric acid and beverages. The soft drink caused a significantly higher decrease in microhardness compared with the other beverages. The black tea caused a significantly higher reduction in microhardness than the mineral water, UHT milk and soymilk, but lower than the soft drink. Among the analyzed beverages, the soft drink and the black tea caused the most deleterious effects on dental enamel microhardness.

Key Words: dental enamel, tooth erosion, hydrochloric acid, *in vitro* study.

INTRODUCTION

Dental erosion or erosive tooth wear is a multifactorial condition (1,2), characterized by the loss of hard tissue by chemical corrosion and dissolution by acids of non-bacterial origin (3). The origin of these acids is either extrinsic (dietary and environmental) or intrinsic (gastrointestinal) (4).

In the dental clinic, the lesions of dental erosion have been commonly found in patients with regurgitation (1), as in gastroesophageal reflux disease, or conditions with recurrent vomiting, such as anorexia and bulimia nervosa (5).

Frequent intake of acid beverages poses risk of erosion damage (6-8) in primary and permanent teeth (6).

Acid juices (3,6), soft drinks and carbonated beverages show erosive effects (7). The chemical composition of a beverage is related to dental erosion (9) and contains citric acid, which is a strong chelator of tooth minerals and has been linked to the erosive effect of beverages (7,10).

On the other hand, a content of calcium, phosphate and fluoride in beverages appears to be an important factor associated with potential erosion (7). Beverages enriched with calcium showed a decreased erosive potential (11) and an increased remineralizing capacity (12). However, the addition of varied and different calcium salts could not only modify the erosive potential, but also change the characteristics of original beverages such as flavor and color (11,12). Promoting

the consumption of milk or other beverages as natural sources of calcium could be a more effective and less costly preventive measure rather than supplementation with this mineral.

Calcium is an essential mineral for the structure of bones and teeth (13). The presence of phosphoproteins and calcium in the composition of cow milk could prevent enamel dissolution (14). Drinking milk after an erosive challenge could have a reparative effect on the remineralization of tooth enamel (3,15).

The soybean extract, known as soymilk, presents calcium (13) and protein (16) in its composition. Soy products have attracted interest because of the health benefits attributed to the consumption of this legume (17).

Black tea contains fluoride in its composition (18). The presence of fluoride has beneficial effects on tooth enamel, increasing the resistance to acid dissolution (19).

The demineralization of the enamel surface could be reinforced by successive exposures of the oral environment to constant challenges of both intrinsic and extrinsic acids. Individuals with a history of diseases that involves vomiting or regurgitation may have a decreased microhardness of enamel surface, depending on the type of usually consumed beverages.

The aim of the present study was to evaluate by

an *in vitro* experimental model the effect of beverages on the microhardness of dental enamel subjected to erosive challenge with hydrochloric acid (HCl).

MATERIAL AND METHODS

Experimental Design

The studied factor was the type of beverage in five levels: Sprite® Zero Low-calorie Soda Lime (Companhia de Bebidas Ipiranga, Ribeirão Preto, SP, Brazil; positive control), Parmalat® ultra high temperature (UHT) integral cow milk (Companhia de Alimentos Glória, Votuporanga, SP, Brazil), Ades® Original soymilk (Unilever Brasil Alimentos Ltda., Pouso Alegre, MG, Brazil), Leão® Ice Tea Zero ready-to-drink low-calorie peach-flavored black tea (Companhia de Bebidas Ipiranga) and Prata® natural mineral water (Águas da Prata Ltda, Águas da Prata, SP, Brazil; negative control). The composition, pH and fluoride concentration of the beverages are given in Table 1.

The experiment used 75 bovine enamel specimens distributed among 5 types of beverages (n=15), according to a randomized complete block design. The quantitative response variable was the microhardness (KHN) of enamel.

Table 1. Composition, pH and fluoride concentration of beverages.

Beverages	Composition*	pH**	Fluoride concentration*** (mg/L)
Sprite® Zero Low-calorie Soda Lime	Carbonated water, lemon juice 2.5%, acidulant (citric acid), synthetic aroma identical to the natural, artificial sweetener (sodium cyclamate and sodium saccharin)	2.91	< 0.2
Leão® Ice Tea Zero ready-to-drink low-calorie peach-flavored black tea	Water, black tea extract, acidulant (citric and phosphoric acid), synthetic aroma identical to the natural, conservatives (sodium benzoate and potassium sorbate), artificial sweetener (sodium cyclamate and sodium saccharin), sequestrant (sodium hexametaphosphate), antifoam (dimethyl polysiloxane)	3.02	0.760
Ades® Soymilk	Soy milk, water, sugar, salt, vitamins (A, C, D, E, B2, B6, B12, folic acid), minerals (calcium and zinc), vanilla flavor similar to the natural, stabilizers (gum gellan, sodium citrate, xanthine gum and soy lecithin)	7.35	0.265
Prata® natural mineral water	Fluoridated radioactive mineral water, bicarbonate, sulfate, sodium fluoride, sodium, potassium, magnesium, calcium, chloride, nitrate	6.29	0.220
Parmalat® UHT milk	Standardized milk with 3% fat, stabilizers (sodium citrate, sodium triphosphate, sodium monophosphate and diphosphate disodium)	6.63	< 0.2

*Composition compiled from data supplied by manufacturers on the product packaging. **pH values measured with a pH meter.

***Fluoride concentration values <0.2 are below the sensitivity of the electrode.

Preparation of Enamel Specimens

One hundred enamel slabs (5x4 mm) were obtained from the coronal portion of 50 freshly extracted bovine incisors using a precision cutting machine (Isomet 1000; Buehler Ltd., Lake Bluff, IL, USA), and stored in 0.1% thymol solution at 4°C. At the moment of use, slabs without fissure were embedded individually in epoxy resin (Epoxicure Resin, Buehler Ltd.) with the buccal enamel surface facing up. Grinding and polishing were performed onto 600- and 1200-grit aluminum oxide abrasive papers (Norton - Saint Gobain, São Paulo, SP, Brazil) in a water-cooled polishing machine (Beta Grinder-Polisher, Buehler Ltd.) and subsequently a 0.3 µm alumina suspension (Buehler Ltd.) was used with slight hand pressure only. Thereafter, the specimens were ultrasonically cleaned (Odontobrás Ind. e Com. Ltda., Ribeirão Preto, SP, Brazil) in deionized water for 10 min to remove any residues of the polishing procedures and immersed in artificial saliva.

Initial Assessment and Selection of Specimens

All 100 specimens were individually immersed in 20 mL of artificial saliva (20) for 48 h at 37°C. Thereafter, Knoop microhardness indentations were made using a HMV-2 microhardness tester (Shimadzu, Kyoto, Japan). Three indentations (25 g for 30 s) spaced 500 µm apart were made at a distance of 500 µm from the edge. After

measurements, a total of 75 specimens were selected based on their higher average microhardness values.

Lesion Formation of Erosive Wear

The selected specimens had the buccal surface isolated except for a central circular area of about 5 mm² before individual immersion in 10 mL of 0.01 M HCl aqueous solution (21) for 2 min. Subsequently, the specimens were washed with deionized water, immersed in artificial saliva (20) for 2 h at 37°C, and then rinsed with deionized water and kept in relative humidity until Knoop microhardness readings.

pH Measurement

The pH of beverages was measured in a pH meter (model mPA 210; MS Tecnopon Piracicaba, SP, Brazil) connected to an electrode calibrated with standard solutions of pH 4.0 and 7.0, respectively.

Quantitative Analysis of Fluorides

Determination of fluoride content in solutions was performed using a selective electrode (Orion model 96-09) combined with ionic strength adjustment and pH, using a 0.5 mol/L citrate buffer, with pH 5.50, ratio 1:1 sample/buffer. The concentration (x) was determined from the value of the obtained potential (y) and applied to the linear equation $y=a+b \log x$ using linear regression from the triplicate readings of suitably diluted standard fluoride solutions, where a is the linear coefficient equation (slope) and b is the angular coefficient. Calibration curve was obtained from the reading in mV of a standard solution of sodium fluoride at 25°C. Concentrations of sodium fluoride ranged from 0.2 to 1,000 mg/L (ppmF⁻).

Immersion in Beverages

The 75 previously eroded specimens were

Table 2. Microhardness measurements (KHN) expressed as mean and standard deviation.

Beverages	KHN-1* Initial KHN	KHN-2 KHN after HCl	KHN-3 KHN after beverages	Mean difference
Soda lime	361.42 (29.97)a	227.38 (37.27)a	97.52 (16.75)	-129.86 (38.64)a
Ice tea	368.58 (35.23)a	219.69 (26.42)a	148.51 (23.32)	-71.18 (27.42)b
Soy milk	361.98 (37.52)a	221.80 (35.86)a	235.07 (43.99)	+13.27 (44.59)c
Mineral water	354.39 (33.09)a	231.47 (45.86)a	246.94 (31.85)	+15.47 (33.14)c
UHT milk	364.04 (30.76)a	230.00 (32.72)a	252.52 (32.91)	+22.51 (33.35)c

*Homogeneity of variance with Bartlett test. Different letters indicate statistically significant difference within columns.

randomly divided into 5 groups and subjected to immersion in the beverages (3 trial treatments and 2 control treatments), twice daily for 2 days at room temperature. The immersions were made under constant stirring in orbital shaker (CT155; Cientec, Piracicaba, SP, Brazil), at 50 rpm speed for 1 min in a Becker tip recipient containing 20 mL of each beverage. The specimens remained individually immersed in 20 mL of artificial saliva (20) at 37°C in the intervals between the immersions in the beverages.

Final Evaluation of Microhardness

Final hardness of the specimens was measured using a HMV-2 microhardness tester (Shimadzu, Tokyo, Japan), performed after immersion in the beverages, with the same load and distance previously used for obtaining the initial microhardness readings, as well as after immersion in HCl. Indentations were made parallel to the earlier measures.

Statistical Analysis

The homogeneity of variance in measures of initial hardness (initial KHN) and after challenge with HCl (KHN after HCl) was verified with the Bartlett test. Data from the initial hardness (initial KHN) and after challenge with HCl (KHN after HCl) were analyzed by one-way ANOVA, following the randomized complete block design. The effect of previously eroded enamel in the beverages was considered as the variable response difference measures observed between microhardness after immersion in beverages and the measures after challenge with HCl. Tukey's test compared the effect of beverages. The softwares STATA version for windows (Stata Corporation, Houston, TX, USA) and R version 2.5.1 (R Foundation for Statistical Computing - General Public License, Boston, MA, USA) were used for statistical calculations in this study.

RESULTS

The Bartlett test showed homogeneity of variance in initial microhardness measurements of enamel specimens (initial KHN) and after challenge with HCl (KHN after HCl).

There was no significant differences of enamel microhardness among the 5 groups in the initial KHN ($p=0.8403$). There were also no significant differences

among the microhardness values of the experimental groups after HCl challenge (KHN after HCl) ($p=0.8761$).

The differences between the enamel microhardness of specimens exposed to HCl and to the tested beverages were highly significant ($p<0.00001$). The soda lime caused the greatest decrease in enamel microhardness, significantly higher than the other beverages. The exposure of enamel to low-calorie black tea caused a reduction of microhardness, significantly higher than the exposure to mineral water, soymilk and UHT milk, however less than the one caused by soda lime. There was no difference between cow milk, soymilk and mineral water, which did not cause further decrease of microhardness of enamel previously eroded with HCl (Table 2).

DISCUSSION

Chemical composition of an acidic beverage is clearly an important factor in the hardness properties of enamel (22). This study evaluated the effect of beverages on dental enamel submitted to erosive challenge with HCl. The protocol used for the formation of erosion lesions was adapted from the *in vitro* model described by Messias et al. (23). In order to simulate the contact of the tooth surface with gastric contents resulting from gastric vomiting or reflux episodes, was used 0.01 M HCl (21) instead of orange juice. As HCl is a strong acid (24), the erosive challenge was set for a single immersion, using a shorter time (2 min instead of 5 min) and a less acid solution (10 mL instead of 20 mL).

This study was developed using five commercially available beverages. Sprite Zero was used as the positive control because of its recognized erosive potential (6,7). Cow milk was tested because it presents calcium and casein phosphoprotein (14,15), which should protect enamel demineralization. The soymilk was included because it has calcium and protein in its composition (13,16). Mineral water was used as the negative control because it has no erosive substances in its composition. The presence of fluoride in black tea composition (18) determined its inclusion, and its presentation as a ready-to-drink low-calorie tea was chosen because of its practicality. Analyzing the results of this study, Sprite Zero provided a significantly higher decrease in microhardness than the other beverages. A likely explanation for this fact could be the presence of citric acid and lemon juice in its composition and a 2.91 pH (below the critical). The chemical composition of

a beverage is important to dental erosion (9). Citric acid is added to accentuate the lemon flavor, but it is a strong chelator of minerals and responsible for dental corrosion (10).

The black tea used in this study caused a reduction in microhardness significantly different than the mineral water, soy extract and UHT milk, but lower than the one caused by the soft drink. The presence of citric and phosphoric acids in the composition of the tested ice tea might have contributed to this result.

The presence of fluoride in black tea was measured by quantitative analysis demonstrated in a concentration of 0.760 mg/L, which was unable to remineralize enamel previously subjected to erosive acid challenge with HCl. Fluoride did not provide a protective effect on enamel when present in acidic carbonated beverages (25). The tested black tea contains fluoride, but other ingredients are also present in its composition, such as citric acid and phosphoric acid, which might have contributed to its negative result in terms of decrease of enamel microhardness.

In this study, UHT milk, soymilk and mineral water did not further decrease the surface microhardness of enamel subjected to erosive challenge with HCl. Milk has considerable interest, due to its large consumption and its relation to tooth structure (14). The content of calcium and other minerals present in the composition of milk could promote enamel remineralization (14,15). Milk showed no significant effect in remineralizing, a possible interpretation of this result could be the total exposure time, which was 4 min. This might be an explanation for the fact that UHT milk and soymilk did not remineralize the enamel eroded by HCl.

Under the conditions of this study, it may be concluded that Sprite Zero and Ice tea worsened the microhardness of dental enamel in a stronger manner compared with the other beverages. UHT milk, soymilk and mineral water did not decrease significantly surface microhardness nor were they able to remineralize the previously eroded enamel with HCl. A reduction or replacement of beverages with potential in demineralizing tooth enamel would be an important factor in preventing damage in tooth structure, especially in individuals with disorders that involve contact with the HCl by regurgitation and vomiting.

RESUMO

Este estudo avaliou através de um modelo *in vitro* o efeito de

bebidas no esmalte dental previamente submetido a desafio erosivo com ácido clorídrico. O fator em estudo foi o tipo de bebida, em cinco níveis: Sprite Zero (Coca-Cola® Brasil) controle positivo, leite integral *Ultra High Temperature* (Parmalat®), extrato de soja integral (Ades® Original), chá preto com baixo teor calórico sabor pêssego (Leão Ice Tea Zero Coca-Cola® Brasil) e água mineral Prata® (controle negativo). Setenta e cinco espécimes de esmalte bovino foram distribuídos entre os cinco tipos de bebidas (n = 15), de acordo com delineamento em blocos completos e casualizados. Para a formação das lesões de desgaste erosivo, os espécimes foram imersos durante 2 min em 10 mL de solução aquosa de ácido clorídrico 0,01 M. Subsequentemente foram imersos em 20 mL das bebidas por 1 min, duas vezes ao dia durante 2 dias, em temperatura ambiente. Nos intervalos entre as imersões os espécimes permaneceram em saliva artificial a 37°C. A variável de resposta quantitativa foi microdureza superficial do esmalte. ANOVA e teste de Tukey demonstraram diferenças altamente significativas (p < 0,00001) no esmalte exposto ao ácido clorídrico e às bebidas. O refrigerante provocou diminuição da microdureza superficial significativamente maior do que as demais bebidas. O chá preto provocou diminuição da microdureza significativamente maior que a água mineral, o leite UHT e o extrato de soja, porém menor que o refrigerante. Entre as bebidas analisadas, refrigerante e chá preto demonstraram efeitos mais deletérios sobre a microdureza do esmalte dental.

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