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Cariogenic potential of commercial sweeteners in an experimental biofilm caries model on enamel

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ABSTRACT

Objective: Scarce evidence is available on the cariogenic potential of the widely used commercial sweeteners. The aim of this study was to evaluate the effect of several sweeteners on enamel demineralisation and on the cariogenic properties of *Streptococcus mutans* biofilms in an artificial caries model.

Methods: *S. mutans*-UA159 biofilms were cultured on bovine enamel slabs and exposed to one of the following commercial sweeteners in tablet or powder form: stevia, sucralose, saccharin, aspartame or fructose. Ten percent sucrose and 0.9% NaCl were used as caries-positive and caries-negative controls, respectively. Slabs/biofilms were exposed to the sweeteners three times per day for 5 min each time. After 5 days, biofilms were recovered to determine: biomass, bacterial counts and intra- and extracellular polysaccharides. Surface microhardness was measured before and after the experiment to assess enamel demineralisation, expressed as percentage of surface hardness loss (%SHL). Data were analysed using analysis of variance (ANOVA) and Bonferroni ($p < 0.05$).

Results: All tested commercial sweeteners, except fructose, showed less enamel demineralisation than sucrose ($p < 0.05$). Only saccharine showed less biomass and intracellular polysaccharides than the rest of the groups ($p < 0.05$). Stevia, sucralose and saccharine reduced the number of viable cells when compared with sucrose ($p < 0.05$). All sugar alternatives reduced extracellular polysaccharide formation when compared with sucrose ($p < 0.05$).

Conclusions: Most commercial sweeteners appear to be less cariogenic than sucrose, but still retaining some enamel demineralisation potential. Products containing stevia, sucralose and saccharine showed antibacterial properties and seem to interfere with bacterial metabolism. Further studies are necessary to deepen these findings.

1. Introduction

The detrimental role of sugars, particularly in dental caries is still a matter of controversy.[1] Sucrose has been traditionally considered a highly cariogenic substrate for the oral biofilm. Upon fermentation by oral bacteria, sucrose molecules are transformed into energy and large amounts of acids.[2] Thus, frequent exposures to this carbohydrate create conditions for caries onset by promoting demineralisation. As an additional virulent mechanism, cariogenic bacteria populating the dental biofilm generate exopolysaccharides to create a protective environment against physiological antibacterial mechanisms of the mouth.[3]

Artificial sweeteners are becoming increasingly used to sweeten beverages, such as soda, juice, coffee and tea. In an

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era where obesity and overweight have become a serious health problem with large populations affected,[4] sweeteners arise as a way to replace sucrose consumption to deal with this public-health matter. These products have been considered as safe in a recently published position article from the Academy of Nutrition and Dietetics.[5]

Several research articles have been published claiming a non-cariogenic or an apparent anti-caries potential of sweeteners.[6] Sugar alcohols (polyols), that is, xylitol and sorbitol have been intensely tested for caries prevention, especially in chewing gums. Despite the multiple investigations into a putative anti-caries effect of xylitol or sorbitol as caries-safe sugar substitutes, these products are not the most frequently used by the food industry. Saccharine, a sulphamide, was the first and most used sweetener. From there, several other artificial sweeteners have been introduced over the time. Currently, five non- or low-caloric sweeteners have been approved by the Food and Drug Administration (FDA): aspartame, saccharine, acesulfame potassium, sucralose and neotame. Moreover, five non- or low-caloric sweeteners are generally recognised as safe by the same institution: sorbitol, xylitol, erythritol, tagatose and stevia.[7] Generally assumed as caries-safe, sweetening beverages with sugar substitutes is becoming increasingly popular. One of the most investigated artificial sweeteners is sucralose. In its pure form, sucralose has been regarded as non-cariogenic[8] and when combined with bulking ingredients is less cariogenic than sucrose. Likewise, scarce evidence, mostly in rats, suggests that aspartame would be non-cariogenic.[9] Stevia is a highly used commercial sweetener derived from a plant, but only one study in rats with a controlled diet reports a non-cariogenic effect of the sweetener.[10] Research on the effect on caries of the currently available commercial sweeteners is rather insufficient.

Importantly, most of the available research on a presumptive anticariogenic or non-cariogenic effect of sweeteners comes from the pure chemical compound. Information on the cariogenicity of the sweeteners when in combination with bulking carbohydrates is more limited and may be of importance for enamel and dentine caries. Given the fact that evidence on the caries effect of sweeteners is still inconclusive[11] and that limited data on the cariogenic potential of carbohydrate-containing products have been reported, the aim of this investigation was to test the cariogenic potential on enamel and the effect on *Streptococcus mutans* biofilms of several commercial sweeteners.

2. Materials and methods

2.1. Experimental design

S. mutans UA159 biofilms were grown using a previously described *in vitro* caries model,[12] with modifications; three exposures per day for 5 min, instead of eight exposures for 1 min. Bovine enamel slabs served as substrates for *S. mutans* anaerobic biofilm formation for 5 days. Initial surface microhardness (SH) was assessed and the slabs were randomly sorted into seven treatment groups: (1) 10% sucrose (caries-positive control), (2) sucralose, (3) saccharine, (4) stevia, (5) aspartame, (6) fructose and (7) 0.9% NaCl (caries-negative control). Biofilms were exposed to the different treatments for 5 min three times a day, simulating what can be considered a typical snack-consumption pattern. The culture medium was changed twice a day. Biofilms were separated from the slabs for analysis of biomass, viable bacteria, polysaccharide production and biofilm protein content. Final surface hardness was measured from the enamel slabs and the demineralisation produced throughout the experiment was estimated by the percentage of surface hardness loss (%SHL). Acidogenicity of the biofilms was estimated through medium pH, measured twice a day at each medium change. Samples were coded to allow blind measurements of the treatment groups. The whole experiment was repeated twice with each condition in triplicate ($n = 6$, per treatment).

2.2. Enamel slabs

Bovine incisors were obtained, disinfected with a 5% NaOCl solution and stored in 0.9% NaCl (w/v) until use for no longer than 30 days. Slabs (4 mm × 7 mm × 1 mm) were prepared using diamond discs with a low-speed hand piece and Soflex polishing discs (3M, St. Paul, MN, USA). Initial SH was determined by three indentations 100 μm apart from each other, performed with a Knoop microindenter with a microhardness tester (402 MVD, Wolpert Wilson Instruments, Norwood, MA, USA) at 50 g for 5 s. Only those slabs of SH $340.87 \pm 24.4\ kg\ mm^{-2}$ ($n = 42$) were included to avoid bias derived from using enamel with different initial SH values. Slabs were sterilised with ethylene oxide[13] and covered with ultrafiltered (0.22 μm) pooled human saliva treated for 30 min with a protease inhibitor cocktail, to emulate the acquired pellicle on the enamel that further facilitates *S. mutans* adhesion.[14] Slabs were suspended into the wells of a 24-well plate by means of a specially designed device made of orthodontic wire.

2.3. S. mutans biofilms

Frozen stocks of *S. mutans* UA159 (kindly provided by Prof. J.A. Cury, UNICAMP, SP, Brazil) were reactivated in 1% glucose-containing brain heart infusion (BHI; Merck, Darmstadt, Germany) at 37 °C and 10% CO_2 for 18 h. Slabs were inoculated with *S. mutans* culture (optical density (OD) 0.8 at 600 nm) and 1% sucrose-containing medium to form the adherent biofilm[14] and incubated for 8 h. Slabs were then maintained in BHI supplemented with 0.1 mM glucose for 24 h, which simulates glucose basal concentration in saliva.[12]

2.4. Sweetener application to the biofilms

Sweeteners in tablets or powder available in the Chilean market were used in this study. Although not in their pure state, the type of sweetener (groups 2–6, as indicated above) was that informed by the manufacturer on the label of the product. Besides the sweetener of interest, all the products contained additional bulking components. Manufacturer information on the composition of the products is presented in Table 1. Treatment solutions were prepared according to the product labelling to a concentration equivalent to 2 teaspoons