EFFECT OF SUCROSE OR FRUCTOOLIGOSACCHARIDE GUM CHEWING AND FLUORIDATED DENTIFRICE ON *IN SITU* REMINERALIZATION OF ARTIFICIAL CARIOUS LESIONS

EFEITO DA GOMA DE MASCAR CONTENDO SACAROSE OU FRUTOOLIGOSSACARÍDEO E DO DENTIFRÍCIO FLUORETADO NA REMINERALIZAÇÃO IN SITU *DE LESÕES DE CÁRIE ARTIFICIAIS*

Marília Afonso Rabelo BUZALAF
José Mauro GRANJEIRO
Assistant Professors of the Department of Biological Sciences, Bauru Dental School, University of São Paulo

Luciane HIROTA
Undergraduate student from Bauru Dental School, University of São Paulo

André Sakima SERRANO
Flávia de ORNELAS
Milena Souza GOMES DA COSTA
Dentists, graduated at Bauru Dental School, University of São Paulo

Many studies have demonstrated that the use of chewing gum stimulates salivary flow, thus, enhancing the saliva remineralization potential. Fructooligosaccharides (FOS) are used in some countries as sucrose substitutes, but their cariogenic potential has not been completely elucidated yet. The aim of this study was to evaluate the effect of chewing gum containing sucrose or FOS (Meiji Seika, Japan) and dentifrice containing 1,500 ppm fluoride (as MFP) on in situ remineralization of artificial carious lesions. Non fluoridated dentifrice was used as control. This was a crossover study, with 8 volunteers, on four stages of 14 days. Volunteers used an acrylic resin intra-oral jaw appliance containing 2 bovine enamel blocks with artificial carious lesion. After each stage, surface enamel microhardness (Vickers, load of 200 g) was analyzed. Microhardness results demonstrated that in all groups there was remineralization. The remineralization percentual (±SD, n) was 60.9 (±7.6, n=6); 93.0 (±18.2, n=16); 77.2 (±11.6, n=10) and 93.7 (±17.4, n=16), for control, dentifrice, sucrose and FOS groups, respectively. ANOVA and Tukey's post hoc test ($p<0.05$) revealed significant differences among FOS-gum and dentifrice in respect to control and sucrose-gum groups. Thus, results showed that FOS-gum chewing is as effective as fluoridated dentifrice on in situ remineralization of artificial carious lesions. Since FOS are also benefic to general health, their addition in chewing gums in substitution to sucrose should be considered.

UNITERMS: Remineralization; Chewing gum; Fructooligosaccharides; Dentifrices, Fluoride.

INTRODUCTION

Many studies have been used to study enamel and dentin remineralization on in situ models, in order to detect an increase or a decrease in mineral content during the development of the carious process for small time periods[28].

Since clinical observations comproved that white spot lesions are reversible, remineralization became an important mechanism for prevention and clinical reduction of enamel caries[2,4,5,8,15,17,21,25,26,28,29,37,39-42]. This way, factors that increase salivary flow and

stimulate remineralization have caries protective effect, like chewing gums, oral antiseptics, fluoridated dentifrices and gels. They are considered good alternatives for the control of dental caries, because they enhance the natural process of remineralization[17].

Chewing gum, even when containing sucrose, increases the saliva buffer capacity in consequence of an increased salivary flow, thus diminishing plaque accumulation, maintaining the pH levels and, consequently neutralizing the deleterious effects of acids produced in presence of fermentable carbohydrates from diet[1,8,15-17,26,28-30,36,42].

Fructoligosaccharides (FOS) are a mixture of oligosaccharides consisting of glucose linked to two, three, or four fructose units[19]. FOS are found in a variety of food products, including onion, asparagus root, Jerusalem artichoke tubers, garlic, salsify and leek. They are produced in a commercial scale either from sucrose through the transfructosylating action of fungal fructofuranosidase or from chicory inulin with partial hydrolysis with endoglycosidases[23,32]. Because of their physicochemical properties and sweetening power, FOS are consumed mainly in pastry, confectionery, and dairy products. Their energy value is about one-half that of sucrose[31]. They have been used in Japan and in Europe as functional sugars, because they are considered benefic to health. Since they are not digested in the small intestine, they pass into the cecum unchanged, where they are selectively used by bifidobacteria, increasing their density. This implies in constipation relief and suppression of production of putrefactive substances in the intestine[3,9,24]. Furthermore, the increase in the density of bifidobacteria corresponds to lower levels of reductive enzymes (β-glucoronidase and glycocholic acid hydroxylase) associated with conversion of procarcinogens to carcinogens[6]. In addition, FOS also enhances Ca and Mg absorption and also the ratio of Ca to Mg in rats[33-36], which implies in an enhanced femoral bone volume and mineral concentrations in bone[38].

As FOS are used in many products it is likely that some product remains in the oral cavity after consumption. Hartemink et al.[22] and Linardi et al.[27] showed that FOS are fermented by oral streptococci in a similar extent as sucrose. Some strains of mutans streptococci also form artificial plaque from FOS. However, the plaque formed contains a significantly lower amount of total carbohydrates when compared to that formed at the presence of sucrose[27].

Since dental plaque formed in presence of FOS has a lower carbohydrate concentration, FOS are benefic to general health and chewing gums stimulate salivary flow, it was considered appropriate to evaluate the effect of chewing gum containing sucrose or FOS (Meiji Seika, Japan) and dentifrice containing 1,500 ppm fluoride (as MFP) on *in situ* remineralization of artificial carious lesions.

MATERIALS AND METHODS

Experimental design

The study involved a crossover design performed in four phases of 14 days each. Eight adult volunteers (aging between 18 and 22 years-old) took part in this study, approved by local Ethics Committee, after signing an informed, written consent (Resolution No. 196 from National Health Council, Health Ministry, Brasília, DF, 10/03/1996). They were healthy and showed normal salivary flow.

Enamel blocks (4X4X3 mm) were prepared from bovine incisors. The surface of the enamel blocks was polished to remove a layer of 50 mM (Featherstone and Zero, 1992). Artificial caries lesions were prepared according to WANG et al.[40] Specimens were demineralized by immersion in 0.1 mol/L lactic acid-sodium hydroxide buffer (20 mL/specimen) containing 1% sodium carboxymethyl cellulose, 3 mmol/L calcium, 1.8 mmol/L phosphate, and 0.263 mmol/L F (pH 4.0) at a constant temperature of 37°C for 39 h. The specimens were removed from the buffered acid solution and rinsed thoroughly in double-distilled water. After each specimen was inspected, it was sterilized by exposure to gas Oxyfume-12 (White Martins), constituted of ethylene oxide for 24 h at 39ºC and stored in 100% humidity.

The volunteers wore custom-made acrylic mandibular appliances, each containing two specimens, placed at the region correspondent to the lingual surface of the first inferior molars. Each subject wore the appliance for four separate 14-day periods: control (placebo dentifrice KB-1080-1-29, Kolynos, Brazil, 4 times/day, after meals), fluoridated dentifrice (Sorriso, Kolynos, Brazil, containing 1,500 ppm F as MFP, 4 times/day, after meals), sucrose-gum (Spin, Sukest, Bauru, SP, Brazil, containing 60% sucrose, 4 times/day (after meals) for 20 minutes and placebo dentifrice and FOS-gum (FOS was obtained from Meiji-Seika Laboratories, Japan and added to the chewing gum at the concentration of 60%, instead of sucrose). The gum was chewed 4 times/day (after meals) for 20

minutes and placebo dentifrice was used. A 7-day wash out period was used after each test regimen with the placebo dentifrice. Each subject wore the appliance continuously except during meal times. The test subjects received oral and written information to refrain from using any antibacterial product. Considering that the study followed a crossover design, with the participation of the volunteers in all steps, the subjects didn't receive any instructions regarding their daily diet. All subjects lived in a fluoridated area (0.6-0.8 ppm).

Microhardness evaluation

Microhardness determinations were made on the enamel specimens at three stages: (H1) initial (sound enamel), (H2) after lesion formation, and (H3) after intra-oral exposure.

The specimens were tested using the M-Testor 337, fitted with a Vickers diamond under a 200 g load. Three indentations were performed in consistent patterns in the center of the specimen. The remineralization percentage (a) was calculated as follows:

$$a = \frac{H3 - H2}{H1 - H2} \times 100$$

Statistical analysis

The data were tested for statistically significant differences by ANOVA, and Tukey's post hoc test. A significance level of 0.05 was selected a priori.

RESULTS

Table 1 shows the mean (SD), minimum and maximum values of Vickers hardness during the three phases (H1, H2 and H3), as well as the remineralization percentage (a). Microhardness determined initially and after lesion formation, didn't vary among the groups, as expected. However, when microhardness was determined after intra-oral exposure, there were differences among the groups. The a values (SD, %) were 60.9 (7.6), 93.0 (18.2), 77.2 (11.6) and 93.7 (17.4), for groups control, and that used dentifrice, sucrose-gum and FOS-gum, respectively. Thus, the enamel blocks in all groups suffered remineralization, but in different degrees. ANOVA realized over the a variable revealed a significant difference among groups ($F=15.324$, $p<0.000001$). Tukey's test showed a significant difference among the groups that used FOS-gum and dentifrice in respect to the others. Despite the group that used sucrose gum had a higher a in respect to control group, this difference was not statistically significant ($p>0.05$).

DISCUSSION

In the present study we analyzed the remineralizing potential of a fluoridated dentifrice containing 1,500 ppm F (as MFP) compared to chewing gums containing 60% sucrose or FOS. It was analyzed enamel surface microhardness that is a very sensitive technique for detection of enamel surface softening or hardening and can be applied sequentially to the same specimen before and after treatments[29].

GELHARDS; ARENDS[21] related that the remineralization rate is high at the first two weeks, diminishing gradually for longer periods. In a previous study we tested the effect of sucrose-gum chewing or fluoridated dentifrice on enamel remineralization in situ, but the experimental intra-oral period was only one week. We could detect surface remineralization on specimens subjected to the action of dentifrice or chewing gum, but the a value was very small (around 4%) and we couldn't detect significant differences between the groups that used sucrose-gum and fluoridated dentifrice[20]. That's why in the present study we used an experimental intra-oral period of two weeks. This period showed that the use of fluoridated dentifrice and FOS-gum chewing promoted a higher surface enamel remineralization in respect to control and sucrose-gum chewing. However, mineral deposition in deeper portions of the carious lesions could only be availed by other methods, like microradiography[29] or cross sectional microhardness[10,11]. Many authors agree that mineral deposition is gradative and faster at the surface than in deeper parts of the lesion[5,21,25,30,36]. This could explain why in our previous study[20] we obtained remineralization percentages much smaller than in this study.

The use of chewing gum increases the salivary flow in consequence of masticatory and gustative stimuli. This increase in salivary flow, in the absence of a significant acid production (like happens in gums sweetened with xylitol and sorbitol) increases saliva and plaque pH, the amount and concentration

TABLE 1- Mean (SD), minimum and maximum values of D1, D2 and D3 and remineralization percentage (a) for all experimental group.

Groups	Stage	Vickers hardness		
		Mean (SD)	Minimum	Maximum
Control	H1	278.0 (19.4)	249.0	299.0
	H2	68.8 (10.5)	56.1	81.8
	H3	196.8 (23.9)	162.0	227.0
	α (%)	60.9 (7.6)	54.8	75.7
Dentifrice	H1	288.8 (30.1)	222.0	336.0
	H2	70.7 (10.9)	57.1	92.4
	H3	269.4 (30.9)	192.0	322.0
	α	93.0 (18.2)	63.3	131.3
Sucrose Gum	H1	290.4 (29.7)	249.0	336.0
	H2	73.4 (9.6)	59.1	88.8
	H3	239.0 (18.0)	212.0	279.0
	α	77.2 (11.6)	60.5	100.0
FOS Gum	H1	284.6 (30.7)	210.0	330.0
	H2	72.3 (8.5)	61.1	88.8
	H3	267.1 (15.3)	240.0	294.0
	α	93.7 (17.4)	70.6	144.2

* H1, H2 and H3 correspond to surface enamel hardness measured initially (sound enamel), after lesion formation, and after intra-oral exposure, respectively. For control, dentifrice, sucrose gum and FOS gum, *n* = 6, 15, 10 and 16, respectively.

of secreted calcium. As a consequence of the pH increase, there's an increase in phosphate concentration[15,26].

It was shown by Dawes; Macpherson[12] that salivary flow rates are increased during gum chewing to a peak of about ten times the unstimulated flow rate during the first minute of gum chewing followed by a fairly rapid decrease to a plateau at three times the unstimulated flow rate after 20 min. These authors found no difference in salivary flow patterns between chewing gum of different flavors, which contained either sucrose or sorbitol. In addition, they found that, for the sucrose-containing gums, the salivary sucrose concentration peaked within the first 1 or 2 min and then fell rapidly. Creanor et al.[8] found no difference in the degree of surface enamel remineralization when chewing gums containing sucrose or its substitutes were used, but in this study fluoridated dentifrice was used along with the chewing gums. In addition it is known that the recovery of the plaque pH to resting values is much slower when a sucrose-containing gum is used, when compared to the use of a sugar-free gum[13,14]. In our study, the use of chewing gum containing sucrose promoted a significantly smaller enamel remineralization than

did the use of FOS-containing chewing gum. Furthermore, FOS-containing chewing gum promoted surface enamel remineralization in the same extent as did the fluoridated dentifrice.

There are a few studies regarding the cariogenic potential of FOS and they indicate that *in vitro*, FOS is metabolized by oral bacteria and the consequent acid production causes a decrease in pH similar to that caused by sucrose[22,27]. However one of the negative aspects of sucrose is that it causes the production of extra-cellular polysaccharides that enhance the plaque cariogenic potential. The plaque formed *in vitro* in presence of FOS has a significantly smaller amount of total carbohydrates when compared to the plaque formed in presence of sucrose[27] and this could help to explain the difference in enamel surface remineralization of FOS-gum when compared to sucrose-gum. In addition, in our study we used a non-fluoridated dentifrice when the chewing gums were used. So, the effect on enamel remineralization was caused only by the chewing gum. If we consider that plaque formed in presence of FOS has fewer carbohydrates than that formed in the presence of sucrose, it was expected that the FOS-gum would enhance enamel remineralization better than the sucrose gum. Since FOS are also benefic to general health, their addition to chewing gums should be considered.

RESUMO

Muitos estudos têm demonstrado que o uso de gomas de mascar estimula o fluxo salivar, deste modo potencializando a capacidade remineralizante da saliva. Os frutooligossacarídeos (FOS) são usados em alguns países como substitutos da sacarose, mas o seu potencial cariogênico ainda não foi completamente elucidado. O objetivo deste estudo foi avaliar o efeito de gomas de mascar contendo sacarose ou FOS (Meiji Seika, Japão) e do dentifrício fluoretado (1500 ppm, MFP) na remineralização *in situ* de lesões de cárie artificiais. Dentifrício não fluoretado foi usado como controle. Este estudo foi cruzado, contando com a participação de 8 voluntários, em 4 estágios de 14 dias. Os voluntários usaram um dispositivo de resina acrílica intra-oral mandibular contendo 2 blocos de esmalte bovino com lesão de cárie artificial. Após cada estágio, era medida a microdureza superficial dos blocos de esmalte (Vickers, carga de 200 g). Os resultados mostraram que em todos os grupos houve remineralização. O percentual de reminaralização (±DP, n) foi 60,9 (±7,6, n=6), 93,0 (±18,2, n=16), 77,2 (±11,6, n=10) e 93,7 (±17,4, n=16) para os grupos controle, dentifrício, sacarose e FOS, respectivamente. A ANOVA e o teste de Tukey ($p<0,05$) revelaram diferenças significantes entre os grupos do dentifrício e goma de mascar contendo FOS em relação ao grupo controle e o da goma de mascar contendo sacarose. Assim, os resultados mostraram que a goma de mascar contendo FOS é tão efetiva quanto o dentifrício fluoretado na remineralização *in situ* de lesões de cárie artificiais. Uma vez que os FOS também são benéficos para a saúde geral, sua adição a gomas de mascar, em substituição à sacarose, deveria ser considerada.

UNITERMOS: Remineralização; Gomas de mascar; Frutooligossacarídeo; Dentifrícios; Flúor.

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Endereço para cprrespondência:

Faculdade de Odontologia de Bauru
Universidade de São Paulo
Al. Dr. Octávio Pinheiro Brisolla, 9-75
Cep.: 17012.901 - Bauru - SP