_____

Versão do arquivo anexado / Version of attached file:
Versão do Editor / Published Version

Mais informações no site da editora / Further information on publisher's website:
https://www.forp.usp.br/bdj/bdj12(2)/trab06122/trab06122.html

DOI:.

Direitos autorais / Publisher's copyright statement:

In Situ Study of Sucrose Exposure, Mutans Streptococci in Dental Plaque and Dental Caries

Jaime A. CURY[1]
Silvana B. FRANCISCO[1]
Altair A. DEL BEL CURY[2]
Cínthia P.M. TABCHOURY[1]

[1]Laboratory of Oral Biochemistry
[2]Department of Prosthesis and Periodontology
Faculty of Dentistry of Piracicaba, UNICAMP, Piracicaba, SP, Brazil

The purpose of this study was to investigate the relationship among frequency of sucrose exposure, mutans streptococci levels and dental caries. Adult volunteers took part in this crossover study performed in 4 phases of 28 days each. The volunteers wore intra-oral palatal appliances containing blocks of human dental enamel and dripped 20% sucrose solution onto the dental blocks from 0 to 8 times/ day. After each phase, the colony forming units (CFU) were determined in dental plaque and enamel dental caries was evaluated using cross-sectional hardness. Sucrose frequency had no statistically significant effect on mutans streptococci levels. In the enamel cross-sectional hardness tests, significant differences (p<0.05) in relation to area of mineral loss were observed only when sucrose exposure was 8 times/day. Similar results were obtained when cross-sectional hardness was assessed at each distance from enamel surface.

Key Words: sucrose, dental caries, mutans streptococci, microhardness.

INTRODUCTION

Sucrose has been considered the most cariogenic carbohydrate due to the increased porosity of dental plaque matrix formed in its presence (1,2). In addition to a glucan-rich matrix, in situ dental plaque formed in the presence of high frequency of sucrose exposure shows a low inorganic concentration of calcium, phosphorous and fluoride (3). Considering that sucrose is also a fermentable carbohydrate, the maintenance of a low pH may induce an ecological change in dental plaque, with an increase in mutans streptococci levels (4). Therefore, a high porosity (2), a low inorganic concentration (3) and high levels of mutans streptococci (4) in dental plaque are factors that can explain the high cariogenicity of sucrose. Even though mutans streptococci have been related to dental caries (5), there is no controlled study in the literature investigating the relationship among frequency of sucrose exposure, mutans streptococci levels in dental plaque and dental caries. Therefore, the aim of this in situ experiment was to study the relationship among frequency of sucrose exposure, mutans streptococci counts in dental plaque and enamel dental caries.

MATERIAL AND METHODS

Experimental Design

The study involved a crossover, blind design performed in four phases of 28 days each. Twelve adult volunteers took part in this study after signing an informed, written consent (Resolution no. 196 from National Health Council, Health Ministry, Brazilia, DF, 10/03/1996). The volunteers were healthy and showed normal salivary flow.

Enamel blocks (3 x 3 x 2 mm) were prepared from impacted human third molars stored in 2% formaldehyde solution, pH 7.0, for at least a month. The volunteers wore acrylic palatal appliances containing

four dental enamel blocks each. A 4.0-mm deep space was created in the acrylic appliance, leaving a 1.0-mm space for plaque accumulation (3,6). Dental plaque was formed on the enamel blocks, which were protected from mechanical disturbance by a plastic mesh fixed in the acrylic surface. The volunteers were randomly assigned to the treatments. The appliances were removed and a 20% sucrose solution was dripped onto the enamel blocks either 0, 2, 4 or 8 times/day. After 5 min the appliances were again placed in the mouth. A new appliance was constructed for the volunteers in each phase. A 10-day washout period was allowed after each phase to eliminate possible residual effects from the treatments. During a 10-day pre-experimental period and the 28-day experimental period, the volunteers brushed their natural teeth with non-fluoride toothpaste and drank fluoridated water (0.70 mg/l fluoride). The volunteers received instructions to wear the appliances at all times, including at night, but to remove them during meals. The volunteers received oral and written information to refrain from using any antibacterial or fluoridated product. Considering that the study followed a crossover design, with the participation of the volunteers in all the steps, they did not receive any instructions regarding their daily diet.

Microbiological Analysis

After each phase, the plastic mesh was removed and the dental plaque formed on all enamel blocks was collected with sterilized plastic curettes, 12 h after the last exposure to sucrose solution. The plaque was weighed and transferred to tubes containing 10 glass beads and sterilized phosphate buffer 0.1 M, pH 7.4 (1.0 ml/mg plaque). The tubes were vortexed and the suspension was serially diluted (from 1:10 to 1:100,000) with phosphate buffer. From each dilution, 50 µl of the sample was plated in duplicate on the selective SB 20 agar (7) containing 20% sucrose and 0.2 U bacitracin/ml. The plates were incubated for 48 h at 37°C in anaerobic jars containing a H_2 and CO_2 mixture (Gas Pak BBL System, São Paulo, SP, Brazil). Representative colonies with typical morphology of mutans streptococci were counted using a stereomicroscope.

Analysis of dental caries

The enamel blocks were carefully removed from

the appliances and embedded in acrylic resin. The blocks were longitudinally sectioned through the center and polished for microhardness determination, considering that there is a linear relationship (r = 0.9) between Knoop (KHN) hardness and mineral content in enamel dental caries lesions (8). The indentations were made using Shimadzu HMV-2000 microhardness tester (Kyoto, Japan) with a Knoop diamond and 25-g load for 30 s. The indentations were made at 10, 20, 30, 50, 70 and 90 µm from the outer enamel surface, 6 indentations in the central region of the enamel block, 6 indentations above and 6 below this. The mean values at all 6 measuring points were then averaged. The areas under the curves (KHN x µm) were calculated using trapezoidal rule.

Statistical Analysis

The data were statistically analyzed according to the crossover design and the differences among the mean values were evaluated by the Tukey test (p<0.05).

RESULTS AND DISCUSSION

Table 1 shows the mutans streptococci levels in dental plaque (CFU/mg dental plaque) and enamel mineral loss according to the sucrose exposure. The data show that there was a tendency for higher counts of mutans streptococci in dental plaque and higher enamel mineral loss according to frequency of sucrose exposure. The treatments were not statistically different for bacterial counts, however, mineral loss was statistically greater when sucrose was used 8 times/day.

The main finding of the present investigation

Table 1. Means (± SE) of mutans streptococci in dental plaque and enamel microhardness according to frequency of sucrose exposure.

Sucrose frequency (times/day)	mutans streptococci (10^4 CFU/mg)	Enamel microhardness (KHN x µm)
0	26.72 ± 13.36	26949.2 ± 632.1
2	46.72 ± 30.81	25527.6 ± 785.0
4	102.44 ± 53.34	24000.8 ± 1157.5
8	52.18 ± 21.48	15887.4 ± 2739.2*

KHN, Knoop hardness number. *Statistically different from other treatments (Tukey test: p<0.05).

was a higher mineral loss when enamel was exposed to sucrose 8 times/day. Eight times exposure to sucrose is equivalent to consuming meals or snacks containing this sugar at the same frequency, e.g., 3 meals and 5 snacks or coffee consumption with sucrose. This observation is in agreement with the results of Von Der Fehr et al. (9) and Geddes et al. (10). Nevertheless, those studies only reported that sucrose exposure of 9 times/day induces carious lesions in dental enamel, but did not compare dental caries development according to different sucrose frequencies. In the present study, it was possible to observe enamel mineral loss according to increasing frequencies of sucrose exposure (0 to 8 times/day).

Dental plaque formed with sucrose exposure of 8 times/day has two unique characteristics, which would be responsible for its cariogenicity. First, sucrose is responsible for a dental plaque rich in insoluble glucans (11) that increase its porosity (1,2). This increased porosity facilitates the diffusion of the substrate, which is metabolized by acidogenic microorganisms producing organic acids and resulting in a low pH in the deepest regions of dental plaque (12). A significant increase in insoluble polysaccharides was reported only when dental plaque was formed in the presence of high (8 times/day) sucrose exposure (3). Second, in the presence of high frequency of sucrose, a low concentration of calcium, fluoride and phosphorous ions is found in dental plaque (3). Inorganic composition of dental plaque is considered important in caries development (13) and these biochemical changes in dental plaque formed in the presence of high sucrose exposure have been confirmed (14).

A significant 41% of enamel mineral loss was observed (Table 1) when dental plaque was formed in the presence of sucrose 8 times/day in relation to control (no exposure to sugar). When sucrose was used 2 or 4 times/day, the percentage was 5% and 11%, respectively, which were not statistically different from the control. These data suggest that fluoride from the drinking water reduced enamel mineral loss when sucrose was used 2-4 times/day. However, mineral loss was not avoided indicating that it would be necessary to disrupt dental plaque regularly to control caries development. In addition, these data show the limitations of fluoridated water to control caries when sucrose is used 8 times/day in the absence of oral hygiene.

An increase of mutans streptococci would be expected according to sucrose exposure considering that a frequent low pH in dental plaque would be an ecological factor for its growth (4). However, statistical differences in numbers of mutans streptococci in relation to sucrose exposure was not observed. Even though De Stoppelaar et al. (15) and Staat et al. (16) found a positive correlation between the sugar content of the diet and the level of mutans streptococci in dental plaque, Scheie et al. (17) found little difference in the prevalence of Streptococci mutans in 3-week plaque among subjects submitted to high and low sucrose diets. In addition, according to Carlsson (18), sucrose did not affect mutans streptococci levels in dental plaque in humans, and Macpherson et al. (19) did not find significant differences in total microbial and mutans streptococci counts between normal and sucrose plaque. These data suggest that other factors may be more important than the number of these microorganisms. Recent evidence has been reported about the ability of microorganisms to synthesize extracellular insoluble polysaccharide (20) and a consequent change in dental plaque matrix (3).

We conclude that the increase in enamel mineral loss produced by high sucrose exposure can be better explained by the change in structure and composition of dental plaque matrix produced by mutans streptococci than by the absolute increase in number of these microorganisms.

ACKNOWLEDGEMENTS

RESUMO

Cury JA, Francisco SB, Del Bel Cury AA, Tabchoury CPM. Relação entre exposição à sacarose, estreptococos do grupo mutans na placa dental e cárie dental - estudo in situ. Braz Dent J 2001;12(2):101-104.

O objetivo deste estudo foi investigar a relação entre exposição à

sacarose, níveis de estreptococos do grupo mutans e cárie dental. Voluntários adultos participaram neste estudo cruzado realizado em 4 fases de 28 dias cada. Os voluntários utilizaram dispositivos intra-orais palatinos contendo blocos de esmalte dental humano e gotejaram solução de sacarose 20% sobre os blocos dentais de 0 a 8 vezes/dia. Após cada fase, as unidades formadoras de colônias (UFC) foram determinadas na placa dental e cárie foi avaliada através de microdureza da lesão do esmalte. Freqüência do uso de sacarose não teve efeito estatisticamente significante nos níveis de estreptococos do grupo mutans. Nos testes de microdureza, diferenças significativas (p<0,05) em relação à área de perda mineral somente foram observadas quando exposição à sacarose foi de 8 vezes/dia. Resultados similares foram obtidos quando microdureza da lesão de cárie foi avaliada a cada distância da superfície do esmalte.

Unitermos: sacarose, cárie dental, estreptococos do grupo mutans, microdureza.

REFERENCES

1. Zero DT, Fu J, Anne KM, Cassata S, McCormak SM, Gwinner LM. An improved intra-oral enamel demineralization test model for the study of dental caries. J Dent Res 1992;71:871-878 (special issue).

2. Van Houte J. Role of micro-organisms in caries etiology. J Dent Res 1994;73:672-681.

3. Cury JA, Rebello MAB, Del Bel Cury AA. In situ relationship between sucrose exposure and the composition of dental plaque. Caries Res 1997;31:356-360.

4. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 1994;8:263-271.

5. Emilson CG, Krasse B. Support for and implications of the specific plaque hypothesis. Scand J Dent Res 1985;93:96-104.

6. Benelli EM, Serra MC, Rodrigues Jr AL, Cury JA. In situ anticariogenic potential of glass ionomer cement. Caries Res 1993;27:280-284.

7. Davey AL, Rogers AH. Multiple types of the bacterium Streptococcus mutans in the human mouth and their intra-family transmission. Arch Oral Biol 1984;29:453-460.

8. Featherstone JDB, Ten Cate JM, Shariati M, Arends J. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. Caries Res 1983;17:385-391.

9. Von Der Fehr F, Löe H, Theilade E. Experimental caries in man. Caries Res 1970;4:131-148.

10. Geddes DAM, Cooke JA, Edgar WM, Jenkins GN. The effect of frequent sucrose mouthrinsing on the induction in vivo of caries-like changes in human dental enamel. Arch Oral Biol 1978;23:663-665.

11. Newbrun E. Sucrose, the arch criminal of dental caries. J Dent Child 1969;36:239-248.

12. Fu J, Zero DT, Anne KM, Dass A. Effect of plaque thickness on glucose retention and acid production. J Dent Res 1991;70:493 (special issue, abstract 1815).

13. Margolis HC, Duckworth JH, Moreno E. Composition of pooled resting plaque fluid from caries-free and caries-positive individuals. J Dent Res 1988;67:1468-1475.

14. Cury JA, Rebelo MAB, Del Bel Cury AA, Derbyshire MTC, Tabchoury CPM. Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. Caries Res 2000;34:491-497.

15. De Stoppelaar JD, Van Houte J, Backer Dirks O. The effect of carbohydrate restriction on the presence of Streptococcus mutans, Streptococcus sanguis and iodophilic polysaccharide-producing bacteria in human dental plaque. Caries Res 1970;4:114-123.

16. Staat RH, Gawronski TH, Cressey DE, Harris RS, Folke LE. Effects of dietary sucrose levels on the quantity and microbial composition of human dental plaque. J Dent Res 1975;54:872-880.

17. Scheie AA, Arneberg P, Orstavik D, Afseth J. Microbial composition, pH-depressing capacity and acidogenicity of 3-week smooth surface plaque developed on sucrose-regulated diets in man. Caries Res 1984;18:74-86.

18. Carlsson P. Distribution of mutans streptococci in populations with different levels of sugar consumption. Scand J Dent Res 1989;97:120-125.

19. Macpherson LMD, Macfarlane TW, Aitchison TC, Stephen KW. The effect of sucrose application and implantation of mutans streptococci on the microbial composition of three-week experimental plaque - an in situ study. J Dent Res 1990;69:1706-1711.

20. Mattos-Graner RO, Smith DJ, King WF, Mayer MP. Water-insoluble glucan synthesis by mutans streptococcal strains correlates with caries incidence in 12- to 30-month-old children. J Dent Res 2000;79:1371-1377.