Xylitol concentrations in artificial saliva after application of different xylitol dental varnishes

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Received: January 21, 2010 - December 15, 2009 - Modification: February 25, 2010 - Accepted: March 17, 2010

ABSTRACT

Objective: The present study analyzed xylitol concentrations in artificial saliva over time after application of varnishes containing 10% and 20% xylitol. Material and Methods: Fifteen bovine enamel specimens (8x4 mm) were randomly allocated to 3 groups (n=5/group), according to the type of varnish used: 10% xylitol, 20% xylitol and no xylitol (control). After varnish application (4 mg), specimens were immersed in vials containing 500 µL of artificial saliva. Saliva samples were collected in different times (1, 8, 12, 16, 24, 48 and 72 h) and xylitol concentrations were analyzed. Data were assessed by two-way repeated-measures ANOVA ($p<0.05$). Results: Colorimetric analysis was not able to detect xylitol in saliva samples of the control group. Salivary xylitol concentrations were significantly higher up to 8 h after application of the 20% xylitol varnish. Thereafter, the 10% xylitol varnish released larger amounts of that polyol in artificial saliva. Conclusions: Despite the results in short-term, sustained xylitol releases could be obtained when the 10% xylitol varnish was used. These varnishes seem to be viable alternatives to increase salivary xylitol levels, and therefore, should be clinically tested to confirm their effectiveness.

Keywords: Xylitol. Artificial saliva. Dental caries.

INTRODUCTION

The influence of xylitol on the control of risk factors and prevention of dental caries has already been assessed in several studies[1,5-12,16-18,21-24,26]. The outcomes of this strategy seem to depend on the detection of minimal salivary levels of that polyol along time[28]. However, the rapid clearance of xylitol from the oral cavity can explain the short-term increase of salivary xylitol concentrations after the use of vehicles such as candies, chewing gums, dentifrices and solutions[15,16,18,24,25].

High frequencies of intake of xylitol have been employed in most of the studies so that its anticariogenic effects could be observed in clinical trials[1,5-12,14,16-18]. However, these protocols could cause discomfort to the patient and strongly rely on compliance[25], which turns difficult the use of xylitol in daily clinical practice. Thus, the need of vehicles that could allow sustained xylitol release along time has been recognized[25,28].

Fluoride[22], chlorhexidine[3], copper[29] and iron[13] have been shown to be successfully delivered when incorporated into dental varnishes because their adherence to tooth surface leads to longer maintenance of these different agents in the oral cavity. However, to our knowledge, no attempt was made to include xylitol into a varnish formulation so far. Hence, the present *in vitro* study aimed at analyzing the xylitol concentrations in artificial saliva along time after application of varnishes containing 10% and 20% xylitol.

MATERIAL AND METHODS

Preparation of bovine enamel specimens

Enamel specimens were obtained from lower bovine incisors. After visual inspection, stained and/or cracked teeth were excluded. Besides, soft tissues were removed from the coronal and root surfaces with the aid of a periodontal curette (Duflex®, SSWhite, Rio de Janeiro, RJ, Brazil). Fifteen enamel specimens (8x4 mm) were obtained after two double sections of the widest portion of the dental crowns, as described by Magalhães, et al.*19* (2008). Subsequently, the specimens were numbered and randomly allocated to 3 different groups (n=5/group), according to the type of varnish that would be applied: (1) 10% xylitol (FGM/Dentscare, Joinville, SC, Brazil); (2) 20% xylitol (FGM/Dentscare) and (3) no xylitol (control; FGM/Dentscare).

Varnish application

Three different varnishes (control, containing 10% and 20% xylitol) were especially manufactured by FGM/Dentscare for the present research. Xylitol concentrations were determined by the maximum incorporation of that polyol into the varnish that would not lead to precipitation. Varnishes used contain colophonium, synthetic resin, thickening polymer, essence and ethanol in their composition (informed by manufacturer). Xylitol was supplied by Danisco (Xylitab® 300, Danisco Brasil Ltda, Cotia, SP, Brazil). Xylitol and control varnishes did not contain fluoride or any other antimicrobial agent.

Enamel specimens were weighed before and after varnish application to standardize the amount used. After a pilot study, 4 mg of the respective varnish were applied on a dry bovine enamel specimen with a microbrush. It was the maximum amount that could be applied considering the area of the specimens. After 10 min, each specimen was inserted into a microcentrifuge tube containing 500 µL of artificial saliva (1.5 mmol/L $Ca(NO_3)_2.H_2O$; 0.9 mmol/L $Na_2HPO_4.2H_2O$; 150 mmol/L KCl; 0.1 mol/L $H_2NC(CH_2OH)_3$ (TRIS); 0.05 µg/mL NaF, pH 7.0)[30].

Times of immersion in artificial saliva

Sample size and times of immersion of the specimens in artificial saliva were chosen based on results of a pilot study (data not shown). After 1, 8, 12, 16, 24, 48 and 72 h from the first immersion, each specimen was removed, washed with deionized water and placed into another microcentrifuge tube containing 500 µL of fresh artificial saliva at room temperature. Saliva samples were frozen until colorimetric analysis.

Analysis of xylitol in artificial saliva

The analysis of xylitol concentrations in artificial saliva was performed by a colorimetric method, using a spectrophotometer (Ultrospec 2000 UV/Visible Spectrophotometer, Pharmacia Biotech, Cambridge, USA) and an enzymatic kit D-Sorbitol/Xylitol (Boehringer Mannheim, R-Biopharm, Darmstadt, Germany). A standard curve was obtained in triplicate using five different amounts of xylitol (0.5, 2.0, 5.0, 8.0 and 10 µg). The absorbance was read at 492 nm. A mathematical equation determined by the manufacturer of the enzymatic kit was used to convert the values of absorbance into xylitol concentration (mg/L). All samples were analyzed in duplicate and the mean repeatability of the readings was 98.6%.

Statistical analysis

Graph Pad InStat version 3.0 for Windows and Graph Pad Prism version 4.0 for Windows (Graph Pad Software Inc., San Diego, USA) were used. Since data presented a normal distribution (Kolmogorov-Smirnov test) and homogeneity (Bartlett test), they were analyzed by two-way repeated-measures ANOVA followed by Bonferroni's *post hoc* test for individual comparisons.

RESULTS

Regardless of the time of immersion, xylitol could not be detected in artificial saliva samples of the control group. Consequently, data obtained only after application of xylitol-containing varnishes are presented.

Mean xylitol concentrations in artificial saliva ranged between 63.4±2.7 mg/L (1 h) and 32.7±0.9 mg/L (72 h) after application of 10% xylitol varnish and between 169.0±1.5 mg/L (1 h) and 12.7±0.4 mg/L (72 h) after application of 20% xylitol varnish (Figure 1). Significant differences were detected for the variables *varnishes* ($F=33$, $p=0.0004$) and *times of immersion in saliva* ($F=2,466$, $p<0.0001$), as well as a significant interaction between these criteria ($F=1,486$, $p<0.0001$).

In overview, a reduction of xylitol concentration was observed along time for both varnishes, except for 48 h after immersion in artificial saliva. Nevertheless, a greater reduction could be noticed after application of 20% xylitol varnish than after application of 10% xylitol varnish (Figure 1). In the short-term (up to 8 h), xylitol concentrations in saliva were significantly higher after application of 20% xylitol varnish in comparison to its counterpart ($p<0.001$). This relationship was inverted after 12 h from application of the xylitol varnish during the remaining time ($p<0.001$).

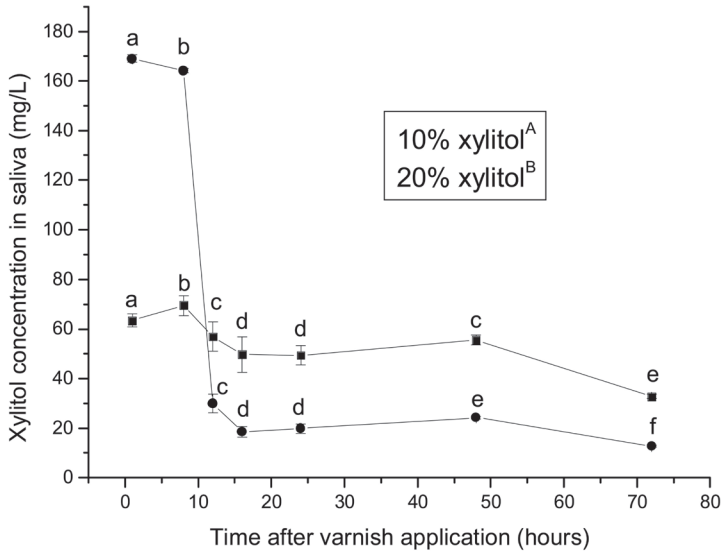

Figure 1- Mean accumulated xylitol concentrations (mg/L) in artificial saliva over time after application of 10% and 20% xylitol varnishes. Bars indicate SD (n=5). Distinct uppercase letters indicate significant differences between the varnishes. Distinct lowercase letters indicate significant differences among time points for each varnish (two-way RM ANOVA, $p<0.05$)

DISCUSSION

When the use of xylitol is aimed to prevent dental caries, important parameters such as frequency of administration, dose-response, and minimal salivary concentration must be regarded. However, such clinical conditions have not been established yet[12,14,20,23]. The amount of 5 g of xylitol, divided in at least 3 times *per* day, is supported for a considerable number of studies as the minimum clinical effective dose to achieve prevention of dental caries. On the other hand, some studies were successful in using lower doses and/or frequencies of that polyol[26]. It has been recently reported that 0.01% xylitol was able to inhibit *in vitro* growth of *mutans streptococci*[28], but the extent to which this information can be transferred to the clinical situation demands further investigation.

Randomized clinical trials normally use daily xylitol amounts that vary between 2 and 15 g, divided in 3 to 5 times *per* day, delivered mainly by chewing gums[6,9-12,20]. However, this protocol can be considered cumbersome[25]. It is desirable to develop new vehicles able to promote a sustained release of xylitol in saliva along time. Hence, xylitol-containing varnishes were formulated and tested in the present study. Dental varnishes can be used in lower frequencies and mastication is not required for their effectiveness which improves patient's compliance. Additionally, the varnishes were manufactured with a fruit flavor to make them more suitable for babies and young children, compared to chewing gums or solutions[4].

It also has been recognized that the frequency of use of xylitol seems to be more important in the prevention of dental caries than the amount of that polyol[24,27,28]. Xylitol is rapidly removed from the oral cavity after application of the vehicles commonly available. Lif Holgerson, et al.[15] (2006) observed that salivary xylitol levels can be significantly increased up to 16 min by mastication of chewing gums containing 1.32 g of xylitol or by mouth rinsing with 10% xylitol solution. Tablets, candies and dentifrices were able to enhance salivary levels of that polyol up to 8 min after be used. Accordingly, the varnishes might have a great advantage over the other vehicles.

This pioneer study evaluated the potential of a newly developed varnish to release xylitol in a medium that resembles the composition of natural saliva along time. Therefore, a positive control group with xylitol delivered via another vehicle, such as chewing gum, was not included. Bovine enamel specimens were immersed in artificial saliva and they were kept under gentle agitation to simulate the movements that occur in the mouth. Conditions as salivary flow and temperature of the oral cavity were not simulated.

In this study, the amount of varnish (4 mg) applied on each specimen might contain 40 and 80 mg of xylitol in 10% and 20% varnishes, respectively, which is very low when compared to that is recommended for prevention of dental caries. However, the volume of varnish usually applied in the whole mouth is around 0.2 mL (~200 mg), nearly 50 times larger than that used in the present research. Consequently, it could be clinically expected that around 2 and 4 g of xylitol would be found in the oral cavity after applying respectively 10% and 20% xylitol varnishes. Higher concentrations of 20% xylitol could not be incorporated into the varnishes without precipitation.

Regarding safety considerations, high doses of xylitol ingested by adults and children can cause adverse gastrointestinal effects[2]. Adults can tolerate the intake of up to 200 g of xylitol *per* day, while children can ingest up to 45 g *per* day. Then, even if the xylitol varnishes would be applied on all dental surfaces, the amount of xylitol that could be ingested is completely safe.

We believe that more homogeneous varnishes might provide more sustained xylitol release along time and, perhaps, this hypothesis could explain the present results. Significantly higher xylitol concentrations were released in saliva after application of the 20% xylitol varnish than of the 10% xylitol varnish on a short-term basis (up to 8 h). Thereafter, this trend was inverted from 12 h until the end of the experimental period (72 h). Therefore, the 10% xylitol varnish was able to produce sustained degrees of xylitol release along time. However, both varnishes were not removed from the dental surfaces throughout the time. This situation is not observed under clinical conditions, when dental varnishes are usually removed from tooth surfaces after 6 to 12 h from application. Taking this into account, a better clinical performance of the 20% xylitol varnish might be expected in relation to its 10% counterpart. Before any speculative conclusion, the performance of these varnishes needs to be tested *in vivo* regarding xylitol release and their caries-preventive effect.

CONCLUSIONS

Considering the limitations of this pioneer *in vitro* study, it can be concluded that sustained xylitol releases can be obtained in artificial saliva after application of 10% xylitol varnish, although 20% xylitol varnish released larger amounts of that polyol in the short-term. These varnishes seem to be viable alternatives to increase salivary xylitol levels and, therefore, they should be clinically tested to confirm their effectiveness.

ACKNOWLEDGMENTS

The authors are grateful to DANISCO Brasil Ltda (Cotia, SP, Brazil) for providing us with the xylitol and CAPES for financial support.

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