Polyol-combinant saliva stimulants: a 4-month pilot study in young adults

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Several studies indicate that xylitol (X) consumption is associated with certain biochemical changes in dental plaque and whole saliva. In making X-containing saliva stimulants more cost-effective and palatable, manufacturers may use maltitol syrup (MS, which normally contains some sorbitol and higher polyols) or polydextrose (PD, a polysaccharide molecule with a mass >22 kDa) as bulking agents. Combinations of X with MS and PD have not been tested regarding their salivary effects. One hundred and eighty-eight young subjects (mean age, 22 years) of both sexes were divided into three groups of equal size for a 4-month study. The subjects in one group used X–MS dragées (in 7 daily episodes; 8 g X per day), while the subjects in another group used X–PD dragées in as many daily episodes (8 g X per day). Subjects in the third (comparison) group did not receive saliva stimulants. Paraffin-stimulated whole saliva samples were collected at baseline, after 2 months, and at endpoint. The usage of X–MS was associated with a significant ($P < 0.05$) reduction in the salivary sucrase activity. After 4 months, the activity of enzymes hydrolyzing Nα-benzoyl-DL-arginyl-*p*-nitroaniline was significantly reduced in all groups, while the levels of free sialic acid were reduced in group X–PD only ($P < 0.05$). These salivary changes most likely reflected microbial shifts in the oral cavity and suggest that information from saliva studies may be of avail when deciding which bulking agents should be used in xylitol-based saliva stimulants. □ *Dental caries; polydextrose; saliva; xylitol*

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Several studies have shown that dietary polyols (sugar alcohols) and sucrose affect the composition and metabolism of human whole saliva and dental plaque differently (1–11). These differences stem from the ready fermentability of dietary sucrose by microorganisms present in the mouth, compared with the generally slow and different type metabolism that the polyols are subjected to in the human oral cavity. For instance, consumption of a xylitol diet or xylitol chewing gum has been shown to be associated with significantly reduced activity levels of the salivary enzyme sucrase (referred to as invertase in some studies) (1–3).

Xylitol chewing gum and other chewable xylitol products (e.g. dragées) have frequently been used to stimulate saliva in laboratory experiments and clinical studies. Owing to the physical and chemical characteristics of the xylitol molecule, xylitol can be used in combination with other polyols or carbohydrates to make such products palatable. Although it is possible to make so-called all-xylitol products (where the entire carbohydrate portion of the product consists of xylitol), there may be additional reasons for using xylitol in combination with bulk sweeteners. One of the reasons is the relatively high price of xylitol.

The confectionery industry has frequently used maltitol syrup (MS, a hydrogenated mixture of starch hydrolysates mostly containing maltitol) and polydextrose (PD, a randomly bonded condensation polymer of D-glucose), in combination with sucrose and other sweeteners. There is no information on the effects on saliva of a xylitol–MS combination (X–MS) as compared with a xylitol–PD-based (X–PD) saliva stimulant. The objective of this pilot study was to investigate the effect of 4-month usage of X–MS and X–PD saliva stimulants (formulated into dragées) on some biochemical properties of human whole saliva. Two Orion Diagnostica test kits (for the assay of lactobacilli and mutans streptococci) were tested regarding their usability in this type of study.

Materials and methods

Subjects and general study design

The subjects of the study were college and high school students of both sexes who lived in the city of Tartu, Estonia. Several months before the study, volunteer subjects within the available student body were searched for by means of advertisements, circulars, and interviews. In January 1995, 188 subjects (mean age, 22 ± 2.2 years; 75% females) were available for baseline examinations. After these examinations, the subjects were randomly assigned to three groups: an X–MS group, an X–PD group, and a comparison group that did not receive saliva stimulants. The subjects were reexamined 2 and 4 months later. At each examination the subjects provided a

paraffin-stimulated whole saliva sample for microbiologic and biochemical tests. No attempts were otherwise made to affect the subjects' oral hygiene practices or their dietary regimens.

Saliva stimulants and their usage

The tested products were polished, pellet-shaped dragées (mean weight, 1.15 g) that were packed in blank, number-coded boxes containing 14 pellets each. The subjects were advised to use the contents of one box each day. The dragées were manufactured and packed by Huhtamäki Oy Leaf (Turku, Finland). The X–MS dragées contained 50% xylitol and 50% MS. The latter (Finmalt L; Xyrofin, Kotka, Finland) contained 62%–70% maltitol, max. 8% sorbitol, and 22%–37% other hydrogenated saccharides. The X–PD dragées contained 50% xylitol and 50% polydextrose (Litesse Improved Polydextrose FCC®, Pfizer, New York, N.Y., USA). The latter was reported to contain max. 4% glucose, max. 2% sorbitol, max. 4% 1,6-anhydro-D-glucose, and a minimum of 90% PD. The mass of the PD molecule was given as >22 kDa. The product contains some citric acid (exact amount not reported). The two types of dragées were identical in shape, color, odor, and appearance, and their taste differences were virtually indiscernible. However, owing to the use of two different raw materials (MS and PD), there was a concomitant difference in the dissolution rate of the MS and PD tablets in saliva (see below).

At the first visit the subjects received a 2-month supply of dragées and a diary for recording the daily usage of dragées. The diary was also used to enter information on the daily intake of sweet items and fruits. The subjects were instructed to use two dragées after breakfast, at 1000 h, after lunch, at 1500, 1800, and 2000 h, and at bedtime (after oral hygiene), for a total of seven daily usage episodes. The subjects were asked to let the dragées dissolve slowly in saliva. At the second visit the diaries were retrieved, and each subject was given a new 2-month supply of dragées and a new diary. At the third visit the diaries were retrieved. On the basis of the chemical composition of the dragées, the daily xylitol consumption per subject in both treated groups was estimated to be 8 g. Thus, the total polyol usage in the X–MS group was about 16 g.

Blinding

The primary purpose of the study was to compare MS and PD as possible bulking agents to be used in combination with xylitol in dragée-type saliva stimulants. The subjects and the researchers involved in clinical, laboratory, and statistical procedures were not aware of the product assignment or the formation of the cohorts. Consequently, this portion of the study was double-blind. The subjects in the third (comparison) group were included in the study merely to provide information on oral microbiologic and biochemical parameters of saliva of untreated subjects of similar age, dietary habits, living conditions, and other characteristics. However, the masking of all analytic procedures applied also to the comparison subjects.

Sample collections and performance of saliva tests

The subjects were requested to refrain from all oral hygiene procedures in the morning of the examination day and on the preceding night. At each visit the subjects were first tested for salivary levels of mutans streptococci using the Orion Diagnostica (Orion, Espoo, Finland) strip test, followed by the collection of a 5-min sample of whole saliva using paraffin stimulation. The flow rate was recorded. An aliquot of the sample was immediately used for a test of salivary lactobacilli using Orion Lactobacillus test kits. The rest of the sample was divided into suitable portions for the analysis of protein and free sialic acid, and for the determination of the salivary activity of sucrase and a peptidase (BAPNA-peptidase, an enzyme or a group of enzymes hydrolyzing Nα-benzoyl-DL-arginyl-*p*-nitroaniline; this enzyme may resemble *Escherichia coli* and *Treponema denticola* oligopeptidase B, EC 3.4.21.83 (12)), and has been suggested to be present in elevated amounts in whole saliva and/or dental plaque of patients with periodontal disease and impaired oral hygiene (see below). A separate aliquot was used for standard microbiologic studies at the Tartu University Institute of Microbiology. The biochemical analyses of saliva were carried out simultaneously at two locations: Tartu University Biochemistry Institute and the University of Michigan School of Dentistry, Ann Arbor, Mich., USA.

The supernatant fluids of whole saliva were analyzed for protein using the biuret reaction. Free sialic acid was determined using the thiobarbituric acid procedure. Sucrase was assayed by means of the neocuproine (2,9-dimethyl-1,10-phenantroline HCl) method to measure the amount of reducing sugars formed from sucrose, using D-glucose as standard (3). The peptidase procedure and all of the above methods have been described in detail elsewhere (3, 12). The rationales for choosing these procedures for the study have been discussed (3).

Statistical procedures

The significance levels between means (at baseline, after 2 months, and at endpoint) were first studied using paired and independent *t* tests. The significance levels were finally studied by means of ANOVA, simultaneously comparing all three groups at different time points, and each group over time. The results shown are based on the use of ANOVA. Because the subjects expectedly showed heterogeneity at baseline in salivary analyses, it was considered justified to use each subject's baseline values as his or her own controls. When testing the biochemical data, the values obtained at the two biochemistry laboratories (Tartu and Ann Arbor) were combined and treated as one set of data.

Results

General observations

Of the 188 subjects examined at baseline, 162 (86%) visited the examination clinic and received their second supply of dragées after 2 months. The number of subjects available at the third visit was 156 (83%). The reasons for dropping out were related to study fatigue and unknown factors. Two subjects (one in each of the treated groups) dropped out owing to subjectively experienced stomach problems. The number of dropouts did not differ significantly between groups. The experimental groups did not differ with regard to the frequency of daily usage of sweet items and fruits.

Owing to the use of different raw materials in the tablets (MS or PD), the dissolution rate of the tablets in the mouth was studied in a group of seven adults (age, 30–60 years; three women, four men). The subjects let the tablets dissolve in saliva in conditions similar to those used in the study proper, and the rate of dissolution was recorded. The subjects were advised not to bite or chew the tablets. The dissolution rate of the X–MS tablets was 5.2 ± 3.2 min, while the dissolution rate of the X–PD tablets was 4.1 ± 2.1 min. The pH values of whole saliva did not differ in samples obtained after the tablets had totally dissolved.

Biochemical and physiologic observations

The results from the measurements of whole saliva flow rate and salivary protein concentrations are shown in Table 1. The values did not change significantly between groups or over time, except that the protein levels were slightly reduced at endpoint in group X–PD. Related, but not significant, changes were also observed in other groups.

The results from enzyme measurements were calculated both as specific activities and per mL of saliva. For brevity, only the former are shown. Group X–MS displayed significantly reduced sucrase activity after 2- and 4-month treatment (Table 2). The sucrase activities did not differ in the comparison group or the X–PD group. Both ways of expressing enzyme activity showed a similar trend.

The peptidase activity reduced in all groups by the end of the treatment, although the reduction was largest (38%) after 4 months in group X–PD (Table 2). The concentration of free sialic acid in whole saliva (calculated per mL) reduced significantly—after 4 months—in group X–PD. The results were similar when the concentrations were calculated per mg protein (not shown).

Microbiologic observations

The treatments had no significant effect on the levels of the mutans streptococcus scores (mean scores for all subjects, 1.7–2.1) and on the $\log_{10}$ lactobacillus scores (mean scores, 4.2–4.6), as determined with the Orion Diagnostica test kits (Table 3). The use of simple microbiologic test kits was considered suitable in this type of study.

Table 1. Whole saliva flow rates and salivary protein levels (mean and standard deviation, *s*) at baseline, after 2 months, and at endpoint (4 months)

	Baseline		2 months		Endpoint	
Experimental group	Mean	*s*	Mean	*s*	Mean	*s*
Flow rate (mL/min)*						
X–MS	1.23	0.57	1.31	0.57	1.35	0.58
X–PD	1.21	0.55	1.26	0.53	1.30	0.53
Comparison group	1.36	0.62	1.36	0.56	1.38	0.53
Protein (mg/mL)						
X–MS	0.93	0.27	0.91	0.27	0.90	0.33
X–PD	0.97	0.30	0.90	0.30	0.84	0.30†
Comparison group	0.92	0.30	0.90	0.26	0.86	0.25

* The values were calculated from 5-min saliva collections. The number of subjects was 49 in each experimental group. These subjects visited at all three examinations.
† ANOVA showed this endpoint value to differ significantly from the baseline value.

Table 2. Activity levels of sucrase and BAPNA-peptidase and concentration of free sialic acid in whole saliva (mean and standard deviation, *s*) at baseline, after 2 months, and at endpoint (4 months)*

	Baseline		2 months		Endpoint	
Experimental group	Mean	*s*	Mean	*s*	Mean	*s*
Sucrase†						
X–MS	23.7	28.8	14.7	12.2‡	15.7	15.4‡
X–PD	17.2	11.7	18.0	13.6	18.3	13.5
Comparison group	16.9	19.8	19.8	19.0	20.8	19.7
BAPNA-peptidase†						
X–MS	0.15	0.13	0.14	0.08	0.10	0.06‡
X–PD	0.19	0.22	0.13	0.09‡	0.12	0.07‡
Comparison group	0.16	0.11	0.15	0.06	0.12	0.05‡
Free sialic acid†‡						
X–MS	8.9	5.5	8.6	6.9	8.5	7.2
X–PD	11.0	8.9	9.9	7.3	7.7	5.2‡
Comparison group	9.8	7.2	9.7	7.1	9.6	5.7

* The values are based on 49 subjects in each experimental group. These subjects visited at all three examinations.
† Enzyme activity is given in nmol/min/mg, and the concentration of free sialic acid in μg/mL.
‡ Significantly different from baseline ($P < 0.05$). ANOVA showed that the 2- and 4-month values did not differ significantly.

Discussion

The study showed that the salivary sucrase activity decreased in group X–MS. Several studies have shown that salivary sucrase activity may be used as a criterion for the cariogenicity of diet. In most experiments where xylitol or other sugar alcohols have been used as a replacement for sucrose, the polyol-consuming subjects have displayed

Table 3. Salivary scores (mean and standard deviation, *s*) of mutans streptococci (SM) and lactobacilli (LB) at baseline, after 2 months, and at endpoint (4 months)*

	Baseline		2 months		Endpoint	
Experimental group	Mean	*s*	Mean	*s*	Mean	*s*
SM Score†						
X–MS	2.1	0.9	2.0	0.8	2.0	0.09
X–PD	1.9	0.9	1.9	1.0	1.7	1.3
Comparison group	2.0	0.8	2.0	0.9	1.9	1.0
LB Score†						
X–MS	4.5	1.2	4.4	1.0	4.4	1.1
X–PD	4.6	1.1	4.4	1.1	4.3	1.2
Comparison group	4.2	1.1	4.3	1.2	4.4	1.2

* The number of subjects was 49 in each experimental group. These subjects visited at all examinations.
† The SM and LB scores were determined by means of Orion Diagnostica test kits according to manufacturer's instructions. ANOVA showed that the groups did not differ at the three examinations, nor did the groups differ over time.

decreased salivary sucrase levels (2, 3, 13, 14). It is possible that the present observations of reduced sucrase levels in the X–MS group (in which the saliva stimulants contained sugar alcohols only) reflect a similar trend. In the other two groups, the subjects either consumed a normal diet (comparison subjects) or received a xylitol product that contained a polymer of a hexose sugar. Thus, these results suggest that the present salivary sucrase response is polyol-specific; that is, the appearance of this response presumes the presence of a high level of polyols in the saliva stimulant. This matter should be elucidated in further studies.

Earlier studies showed that the BAPNA-peptidase activity (12) and the activity of total protease (15) in saliva were significantly reduced in subjects with improved oral health conditions. In the present study the polyol combination X–MS showed a marginally decreasing trend in the salivary BAPNA-peptidase activity. These data do not warrant generalizations; experiments should be performed to verify them as well as the possible significance of this enzyme activity for oral health in general. The levels of salivary free sialic acid(s) were most significantly reduced in the X–PD group. The significance of the free sialic acid levels in saliva is based on the assumption that the sialic acid residues, frequently occupying a terminal position in the oligosaccharide side chains of salivary immunoglobulins, are damaged by the neuraminidase-like enzymes produced by oral micro-organisms. The sialic acid–deficient immunoglobulin molecules lose their biologic activity. Two studies have suggested that the salivary free sialic acid levels reflect the status of oral health (3, 16). Like the salivary proteinase/peptidase activity, the sialic acid procedure as a possible indicator of the oral health status must be evaluated in further studies.

The present study did not include a crossover practice, owing to local, venue-related difficulties regarding the timing of the study. A crossover design in a dental xylitol study may not necessarily be an advantage, however. Several studies have shown xylitol to exert certain long-term effects that would invalidate a crossover study practice (7, 11, 17, 18). Although the assignment of the subjects to the three groups was random, it is possible that the three cohorts were not exactly identical with regard to all relevant factors. The results should also be examined in the light of the relatively short treatment time, although some other studies of equal or even shorter duration have demonstrated significant differences between xylitol and other carbohydrates regarding various salivary indicators. On the other hand, the present subjects were relatively similar with regard to their demographic background, oral health status, oral hygiene, and dietary practices (most subjects were stomatology students). These aspects should increase confidence.

The microbiologic tests failed to demonstrate significant differences between the treatments, nor did they show significant differences between baseline and endpoint. It is possible that the treatment time was too short in this student sample. Furthermore, it is not known to what extent the transition from one season (early winter) to another (spring) during the study, with the possible consequential changes in dietary regimens and salivary physiology, may have influenced the outcome. It is also possible, as demonstrated in an earlier study employing xylitol and sorbitol dentifrices (19), that the present microbiologic procedures were too crude to measure those subtle changes that may take place in the oral biology of subjects who are treated with relatively similar (polyol-based) agents. Certain biochemical tests have turned out to be more sensitive than the present microbiologic procedures. However, these bacteriologic methods were shown to reveal differences between experimental groups that consumed carbohydrate sweeteners with greater chemical differences (2, 3).

Certain physical and chemical differences are inevitable—and often desirable—in products containing different bulking agents, such as MS and PD. The X–PD tablets are somewhat harder, but dissolved more rapidly in saliva, than the X–MS tablets. The extent to which this solubility difference may have affected the present results cannot be determined on the basis of this study. In future experiments the oral clearance of xylitol (and of the bulking agents) should be considered. It is likely, however, that solubility differences did not consistently 'favor' either tablet and do not explain the biochemical salivary results obtained.

In conclusion, the present results suggest that the X–PD and X–MS combinations may not be regarded as dentally harmful (none of the biochemical variables showed an increasing tendency during treatment). The X–MS combination may show some advantage (owing to the decrease of the sucrase levels in this group) provided that these findings can be substantiated in future studies that also determine the clinical effect of X–MS mixtures on dental caries.

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