Effect of xylitol in an enzyme-containing dentifrice without sodium lauryl sulfate on mutans streptococci in vivo

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The aim of this investigation was to compare the effect of an enzyme-containing dentifrice without sodium lauryl sulfate but with addition of xylitol (Zendium Dentine) on mutans streptococci (MS) in saliva and dental plaque with that of the same dentifrice without xylitol. The subjects were divided into a test group, using a dentifrice with 10% xylitol (part A) or 5% xylitol (part B), and a control group, using a dentifrice without xylitol, for 3 months. In part A the MS counts in saliva and plaque were significantly lower in the xylitol group ($n = 50$) than in the control group ($n = 57$) ($P < 0.01$ and $P < 0.001$, respectively). In part B ($n = 89 + 91$), evaluating MS counts in saliva only, no significant difference was found. Thus, this study demonstrated 1) that addition of 10% xylitol to an enzyme-containing dentifrice without sodium lauryl sulfate has an inhibitory effect on MS counts in saliva and dental plaque, and 2) that the inhibitory effect seems to be dose-dependent. □ *Microbiology: plaque; saliva; sugar alcohols; toothpaste*

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Several clinical long-term studies suggest that xylitol may contribute to prevention of dental caries (1–5). Various mechanisms have been suggested for the cariostatic effect of this five-carbon sugar alcohol (6–8). Most oral microorganisms cannot utilize xylitol in their metabolism (9, 10). Xylitol has been found to inhibit both growth and acid production by mutans streptococci (MS)—that is. *Streptococcus mutans* and *S. sobrinus* (11–13). Sorbitol, on the other hand, being a six-carbon sugar alcohol, may serve as a substrate for both these species (10, 14). A decrease of MS in the oral cavity has been reported after frequent consumption of xylitol (15–17), whereas an increase may occur after frequent sorbitol intake (18, 19).

Recently, some investigators have reported that systematic use of a dentifrice containing 10–20% xylitol may reduce the number of MS in saliva (20–22), whereas others have been unable to demonstrate such an effect when using less than 10% xylitol (23, 24). In one of these latter studies (23), only 3% xylitol was used. A possible explanation for the contradictory results obtained by different researchers can thus be that the xylitol content in the test dentifrice has varied to a great extent. Another reason may be that the dentifrices have contained sodium lauryl sulfate (SLS), which inhibits the uptake of xylitol and the formation of xylitol-5-phosphate by plaque bacteria (25, 26). The aim of the present investigation was therefore to compare the effect on MS counts in saliva of SLS-free dentifrices containing 10% and 5% xylitol. When studying 10% xylitol, plaque samples were also included.

Materials and methods

The study consisted of two separate parts (A and B) (Table 1), which had a similar design. Both A and B were carried out double-blind. In part A an additional 2-week follow-up period was included, to investigate a possible sustained effect of xylitol on the MS counts.

Participants

In part A 160 and in part B 309 university students in the city of Kristianstad, Sweden, predominantly women (82%), with a mean age of 30 years, were screened for salivary counts of MS. Those having $>10^3$ colony-forming units (CFU) per milliliter saliva were invited to participate. After signing an informed consent form, the individuals were divided into a test and a control group, to create two matched groups with regard to their baseline MS salivary counts.

Dentifrices

Two types of dentifrices (Table 1) were used, distributed in identical, white (coded) tubes. The basic formula (Zendium Dentine, Household & Personal Care Research, Amersfoort, The Netherlands) was the same in both studies. Thus, all products contained silica as an abrasive agent and 0.15% F (as NaF). The control dentifrice in part A did not contain enzymes (amyloglucosidase and glucose oxidase); the three other formulas, however, contained these enzymes. None of the

Table 1. Composition of the dentifrices. The concentration of some ingredients in the dentifrices (% weight basis) are presented

	Part A		Part B	
	Test	Control	Test	Control
Xylitol	10	0	5	0
Sorbitol (70%)	18	32	12	19
Glycerol (87%)	3	3	9	9
Enzymes*	Yes	No	Yes	Yes
SLS†	No	No	No	No

* Amyloglucosidase and glucose oxidase.
† Sodium lauryl sulfate.

Table 2. Sampling occasions in part A and in part B (S = saliva sample; P = plaque sample)

	Sampling occasion			
Groups	Base line	6 weeks	3 months	Follow-up (2 weeks)
10% xylitol (part A)				
Xylitol group (n = 50)	S, P		S, P	S
Control group (n = 57)	S, P		S, P	S
5% xylitol (part B)				
Xylitol group (n = 89)	S	S	S	
Control group (n = 91)	S	S	S	

products contained SLS. Instead, the mild, non-ionic detergent stearyl ethoxylate was used in both dentifrice formulas. During the 2-week follow-up period (part A) all participants were given the control dentifrice without xylitol, recoded so neither the participants nor the investigator could identify the original codes.

Toothpaste technique

The subjects were asked to use the dentifrices twice daily (after breakfast and immediately before going to bed) for 3 months, to abstain from using other dentifrices, and to maintain their normal dietary habits. Both verbal and written instructions were given on how to use the toothpaste. The dentifrice technique was the same as described by Svanberg & Birkhed (21). Thus, the dentifrice was squeezed over a distance of 1.5 cm on a wet toothbrush and evenly spread on the maxillary teeth, which were then carefully brushed. This procedure was repeated for the mandibular teeth. The subjects were told not to expectorate more than necessary during brushing. Finally, a sip of water, together with the dentifrice foam, was filtered in the dentitition by active cheek movements for 1 min before expectorating. Further mouthrinsing with water directly after the brushing was to be avoided.

Regular contact was kept with the subjects throughout the study (by letter and telephone) to remind them how to use the test dentifrice.

Saliva and plaque samples

All sampling procedures were carried out by the same dental hygienist. An unstimulated whole saliva sample (approximately 3 ml) was collected both in part A and in part B (Table 2). The subjects had been told to refrain from oral hygiene on the morning of the examination day and not to eat or drink anything for 1 h before sampling. One milliliter of saliva was transferred to a vial with 5.7 ml of reduced transport fluid (RTF) (27). Immediately after the saliva sample had been collected, approximal plaque samples were obtained (only in part A, Table 2), in accordance with Lindquist & Emilson (28). Four sterile, triangular wooden (birch) toothpicks (TePe Munhygienprodukter AB, Malmö, Sweden) were inserted into each of four interproximal spaces (15/16, 25/26, 35/36, and 45/46). The four tips (with adherent plaque) were cut off and dropped into the same well in a microtitre plate, containing 0.3 ml of RTF.

The saliva and plaque samples were sent by mail to the Department of Cariology in Göteborg and were processed within 24 h. The samples were homogenized, serially diluted in 0.05 M phosphate buffer with 0.4% (w/v) KCl, and plated on MSB agar (29) for estimation of number of MS. The agar plates were incubated for 48 h at 37 °C in jars filled with 95% N_2 and 5% CO_2. The identification of MS was based on colony morphology as described by Emilson (30). The number of MS in saliva was expressed as CFU per milliliter and in plaque as CFU per sample.

Table 3. Effect of 10% xylitol (part A) on salivary levels of mutans streptococci (log CFU/ml) at base line, after 3 months, and at the follow-up examination (mean ± standard deviation). The changes between base line and 3 months and between base line and follow-up are also given

				Change	
	Base line	3 months	Follow-up (2 weeks)	Base line versus 3 months	Base line versus follow-up
Xylitol group (n = 50)	4.79 ± 0.90	4.28 ± 1.08	4.47 ± 0.94	−0.51 ± 1.06	−0.32 ± 0.94
Control group (n = 57)	4.87 ± 0.72	4.83 ± 0.86	4.72 ± 0.86	−0.04 ± 0.66	−0.15 ± 0.79

* $P < 0.05$; ** $P < 0.01$.

Table 4. Effect of 10% xylitol (part A) on plaque levels of mutans streptococci (log CFU/sample) at base line and after 3 months (mean ± standard deviation). The changes between base line and 3 months are also given

	Base line	3 months	Change
		***	
Xylitol group (n = 50)	3.64 ± 1.94	2.85 ± 2.07	−0.79 ± 1.57
Control group (n = 57)	3.10 ± 1.81	2.69 ± 1.76	−0.41 ± 1.73

*** $P < 0.001$.

Statistical analyses

All MS counts (both in saliva and in plaque) were logarithmically transformed. The changes over time were compared between the two groups by one-way analysis of variance (ANOVA). The individual changes within each group were compared by Student's paired t test. $P < 0.05$ was considered statistically significant.

Results

Effect of 10% xylitol (part A)

Saliva samples. The data are presented in Table 3. After the 3-month period a significant decrease of the MS counts occurred in the xylitol group ($P < 0.01$) but not in the control group; the MS counts differed at the end point between the two groups as well ($P < 0.05$). A difference was also found for the change—that is, base line versus 3 months ($P < 0.05$). At the follow-up (2 weeks later) no significant difference in the MS counts was found between the groups. However, when comparing the data within the groups, still lower MS values were found in the xylitol group compared with base line ($P < 0.05$).

Plaque samples. The data are presented in Table 4. There were no significant differences between the two groups at 3 months. However, within the groups there was a significant decrease in the xylitol group compared with base line ($P < 0.001$), whereas no such difference was found in the control group.

Effect of 5% xylitol (part B)

The data are presented in Table 5. There were no statistically significant differences either between or within the groups during the 3-month period.

Discussion

The first part of this 3-month study (A), evaluating the addition of 10% xylitol to a dentifrice, showed a significant decrease of MS both in saliva and in dental plaque. The decrease could still be detected at the 2-week follow-up examination. Although the primary aim of this study was not to study the effect of enzymes added to a dentifrice, the use of non-enzyme-containing toothpaste as a control in part A and an enzyme-containing toothpaste in part B made it possible to evaluate the effect of enzymes per se. However, the experimental dentifrice in both part A and B contained two ingredients—xylitol and enzymes—that potentially could influence the amount of MS. Thus, the reduction of MS in part A could be explained either by the presence of xylitol or enzymes or by a combination of these two ingredients. However, in part B, evaluating the addition of 5% xylitol, both the experimental and control dentifrice contained enzymes, and no reduction of the MS counts could be detected in any of the groups. The combined results from A and B therefore indicate that the enzymes (amyloglucosidase and glucose oxidase) themselves had no (or only a minor) effect on MS in saliva or plaque. Moran et al. (31) reported that a toothpaste with a similar concentration of enzymes (Zendium) as used in the present study had an effect both on the total streptococcus counts in saliva and on the aerobic and anaerobic flora. The evidence for the inhibitory effect of the enzymes in Zendium and Zendium Dentine on plaque microorganisms is therefore conflicting, as has been pointed out by others (32). Neither Etemadazadeh et al. (33) nor Moran et al. (34) found any effect on plaque formation after the use of this enzyme-containing dentifrice. Thus, it seems most likely that the reduction of MS in saliva and plaque found in the first part of this study (A) was due to the

Table 5. Effect of 5% xylitol (part B) on salivary levels of mutans streptococci (log CFU/ml) at base line, after 6 weeks, and after 3 months (mean ± standard deviation). The changes between base line and 3 months are also given

	Base line	6 weeks	3 months	Change (base line versus 3 months)
Xylitol group (n = 89)	5.67 ± 1.10	5.56 ± 1.28	5.47 ± 1.11	−0.20 ± 0.60
Control group (n = 91)	5.80 ± 0.92	5.67 ± 1.08	5.57 ± 0.93	−0.23 ± 0.60

presence of 10% xylitol in the dentifrice and not to the enzymes amyloglucosidase and glucose oxidase.

A reduction of MS in saliva after the use of a xylitol-containing dentifrice has also been reported in other studies (20, 21). On the other hand, Twetman & Petersson (24) could not demonstrate any definite effect of a 10% xylitol dentifrice in a group of preschool children. The parents in that study were trained to brush the teeth of their children for 2 min twice a day, using approximately 0.5 g of dentifrice. This toothpaste technique may result in a lower xylitol concentration in the oral cavity than in the present study or in the study by Svanberg & Birkhed (21), in which more toothpaste was used and in which the brushing was ended with an active rinse with the toothpaste slurry. The dose of xylitol in the oral cavity and the length of time that xylitol is present in the mouth may be important factors to obtain an inhibitory effect on the MS counts in saliva. In the present study special attention was paid to the dentifrice technique, and this may have led to an increased dose of xylitol being retained for a longer time in the oral cavity, explaining the differences in the results as compared with the study by Twetman & Petersson (24). Another factor that may influence the MS counts in saliva is the amount of sorbitol in the paste. It should be pointed out in this respect that both control dentifrices contained more sorbitol than test dentifrices, especially in part A.

Another factor that may affect the availability of xylitol to the oral microorganisms in situ is the presence of SLS in a dentifrice. Assev & Rölla (25) and Wåler & Rølla (26) recently found that the antibacterial effect of xylitol may be hampered by SLS. In the present study, none of the dentifrices contained SLS. In spite of this, no inhibitory effect of the dentifrice containing only 5% xylitol on the salivary MS counts was found. Petersson et al. (23), also using a relatively low concentration of xylitol (3%), were unable to detect any inhibitory effect on the MS counts. Thus, besides the influence of the toothpaste technique and the presence of SLS in a dentifrice, it is also possible, indeed probable, that the inhibition of MS counts is dose-dependent. Such a dose-response effect on MS has recently been found for xylitol-containing chewing gums (35).

In conclusion, this study demonstrated that 1) xylitol in an enzyme-containing dentifrice without SLS has an inhibitory effect on MS counts, and 2) this inhibitory effect seems to be dose-dependent.

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